COMPARATIVE STUDIES OF GROWTH IN DECIDUOUS AND EVERGREEN TREES

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Introduction

Several decades ago the School of Forestry of the University of Idaho established an arboretum on the campus at Moscow, Idaho. Although located in a prairie region, the majority of the exotic trees planted there have survived and grown well. Possibly this resulted in part from the fact that advantage was taken of the shelter provided by contiguous north-facing and east-facing slopes, but the trees were planted in closed stands and are not irrigated. Thus many species of trees with wide areas in the North Temperate Zone are growing together under a summer-dry climate to which none is native but to which all have become adjusted. The writers looked upon this as an excellent opportunity to make certain comparative studies of growth and related phenomena.

Methods

A new type of precision dendrometer described elsewhere (1) was used to measure radial changes. In all instances the instruments were located on the north sides of the trunks, between 1.0 and 1.5 meters above the ground. Two or three thrifty trees of each species or variety were measured at intervals throughout the calendar years of 1944 and 1945. In general, radial changes of different specimens of the same species corresponded so closely that none of the conclusions reached would be changed if based on the record from only one tree. The use of more than one tree was of most value in enabling interpolations when a single record was lost because of vandalism or other causes.

During the early part of the growing season measurements were made on alternate days, but during less critical periods at intervals of a week or more. On rainy days in any season there are practically no daily reversible changes in radii so that significant measurements can be made at any time of day, but at other times useful observations could be made only under special conditions. During the warm season and especially during the period in which active growth began, measurements were made as early after dawn as possible so that the data would not be affected by temporary diurnal shrinkage resulting from vigorous transpiration. During the cold season only those measurements were used which were made in the morning when air temperature was at approximately freezing or above, in order to minimize the influence of thermal shrinkage which begins to take place when the temperature falls below the freezing-point of water.

Since the aim has been to make qualitative comparisons of radial changes, the latter have been expressed in graphs as percentages of the total increment for each calendar year. This method has the advantage of reducing to a minimum the effects of age, vigor, and competition but has a disadvantage in that the degree of

radial fluctuations in trees which show little total growth is exaggerated in comparison with others. Since the net growth (table 1) has been taken into account in interpreting the graphs, it is thought that the advantages of the percentage method far outweigh this single disadvantage.

TABLE 1

SPECIES AND VARIETIES OF TREES STUDIED AND *NET ANNUAL INCREASES OBSERVED IN RADII*

TREES	NET ANNUAL INCREMENT (MM.)	
	1944	1945
Evergreen conifers:		
Abies concolor (Gord. & Glend.) Hoopes	3.28	3.08
Picea engelmannii Parry	2.31	1.98
P. pungens Engelm.	1.42	1.59
Pinus monticola Dougl.	1.73	1.97
P. ponderosa Laws.	3.78	5.74
P. strobus L.	2.26	1.49
Pseudotsuga taxifolia (Poir.) Britt.	2.63	2.06
P. taxifolia var. glauca (Mayr) Sudw.	2.26	1.78
Deciduous conifers:		
Larix occidentalis Nutt.	3.82	3.11
Deciduous dicotyledons:		
Acer pseudoplatanus L.	5.26	4.16
A. saccharophorum K. Koch	3.01	2.77
Fagus grandifolia Ehrh.	4.93	3.86
Fraxinus americana L.	2.42	2.24
Juglans nigra L.	1.33	1.47
Quercus borealis Michx. f.	5.16	3.90
Robinia pseudoacacia L.	1.47	0.86
Ulmus americana L.	1.28	1.31

ENVIRONMENTAL RECORDS

The instruments of a co-operative station of the United States Weather Bureau are located on the campus on a north-facing slope which is quite comparable with the habitat on which the trees are growing. Daily maximum and minimum air temperatures, daily precipitations in excess of 2.54 mm., and open-pan evaporation records have been extracted from the data collected at this station and are presented in figure 1. Temperature and evaporation records have been plotted for only alternate days through the periods for which there are data. Such simplification makes the graphs more readily comprehensible, yet it brings out the magnitude of diurnal as well as of seasonal fluctuations which are ecologically very important. Also included in figure 1 are soil temperature records taken at a depth of 20 cm. on those dates when tree radii were measured. The soil data are based on the average of one measurement on the north-facing and one on the east-facing slope in the arboretum. Because the trees are growing in a closed stand, soil temperature at any one time is quite uniform, and differences between the two slopes are always very slight. The cyclic daily fluctuation in soil temperature at this depth is very small and influences the data but little, since most measurements were made at approximately the same time of day.

Results and discussion

COMPARISONS BETWEEN DECIDUOUS DICOTS AND EVERGREEN CONIFERS

The following discussion is based entirely upon the eight deciduous dicotyledons and the eight evergreen conifers, for which groups average cumulative growth is presented in figure 2. All reference to the deciduous conifer *Larix occidentalis* is deferred to the subsequent discussion. The averages represent median points within the two groups, respectively.

During the 5-month period from December through April, evergreen conifers showed more response to weather changes than did the deciduous dicots. The radii of the latter varied but little from the midwinter zero point and changed less abruptly than those of the

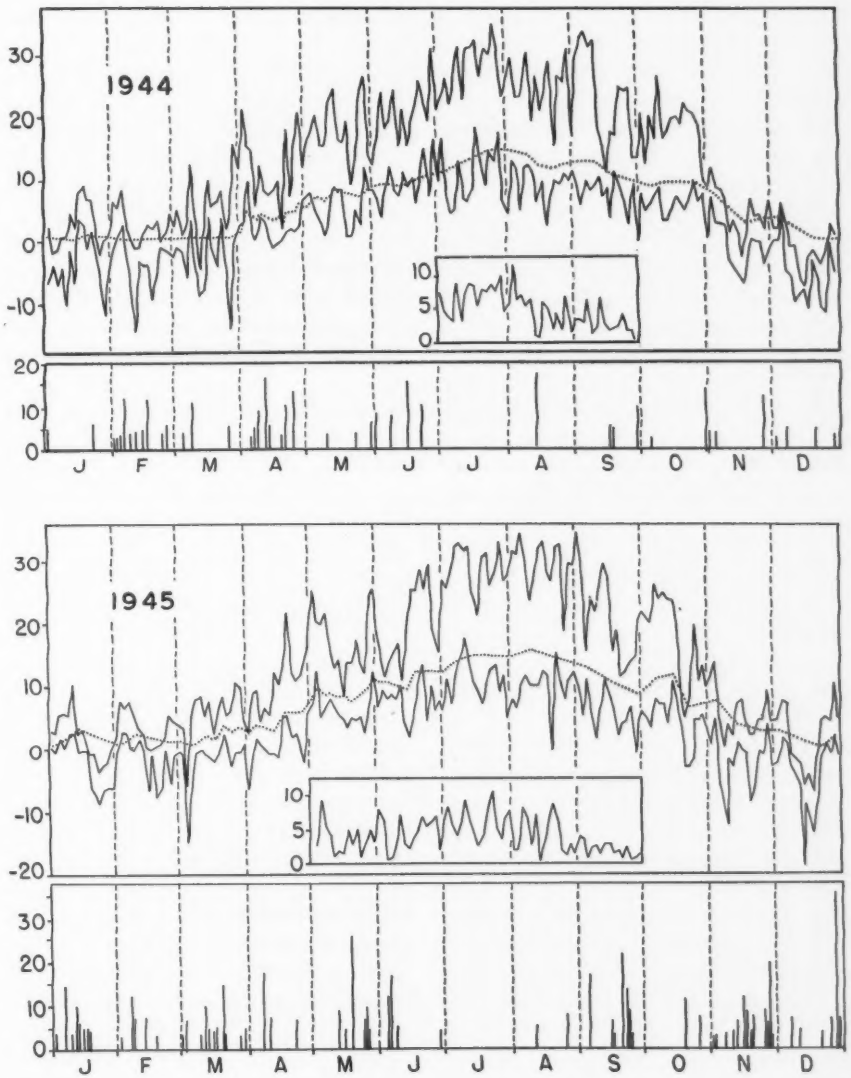


FIG. 1.—Environmental records for 1944 and 1945. For each year: *upper*, maximal and minimal air temperatures, and soil temperature (dotted line) at 20 cm., in °C.; *inset*, open-pan evaporation in mm.; *lower*, precipitation in mm., omitting amounts less than 2.54 mm.

conifers. This difference may be accounted for by the persistent leaves of the conifers, which render these trees very susceptible to shrinkage resulting from vigorous transpiration, and by their softer wood, which may be more responsive to turgor pressure changes.

The data for both years reveal a remarkable identity in the date of beginning of the grand period of growth for the two groups, even though they are unrelated ecologically as well as taxonomically. Both groups grew for a few days in early April, 1944, in response to a brief period of above-average temperatures, and then remained inactive during a fortnight of cool weather.

Except for about a week in May, 1945, there was a distinct tendency for the evergreen trees to grow more rapidly than the deciduous trees during the early part of the grand period of growth, an advantage which they maintained throughout the summer, even though the rate of increase dropped off earlier in the evergreen trees. In general, the growth rates of the two groups were essentially identical during the period of most active enlargement. During July the rate of growth declined rapidly, especially in the evergreens.

Subsequent to the grand period of growth, radii fluctuated considerably but continued to show net increases until midwinter. The curves from midsummer to midwinter are very similar both years for the deciduous trees, but the conifers responded quite differently to the differences in weather in the two seasons. The summer of 1944 was not so dry as that of 1945, but the season of inadequate precipitation lasted through October. In 1945 the rains stopped earlier, but the dry season was broken by a period of rainy weather in early September. Thus, the principal rainless season came earlier in 1945.

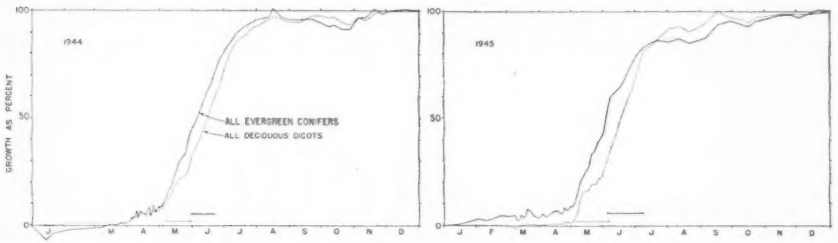


FIG. 2.—Radial changes in stems in percentage of total yearly increment, based on averages of eight species of each group. Horizontal lines explained in text.

The evergreens, which as a group shrank more than the deciduous trees during summer drought, showed their principal response late in the summer of 1944, when the dry season was late, and in 1945 they suffered the most shrinkage earlier in harmony with the earlier occurrence of the dry season.

In the first few months of 1945, a period which in this latitude is generally conceded to be characterized by dormancy, more than 5% of the total annual increment in the evergreens was laid down. This cannot be interpreted as rehydration of tissues that shrank during the preceding summer, for the 1944 record shows clearly that recovery had been complete by the end of the calendar year. It is possible, however, and indeed most

likely, that the January-through-March swelling of the evergreens in 1945 represented belated hydration of tissues formed the preceding summer.

Comparisons of Larix with deciduous dicots and evergreen conifers

The relation of *Larix occidentalis* to the other trees is unique, since it is a conifer and yet has the deciduous habit of the dicots. Although the study of this species was not begun until March, 1944 (fig. 3), this date was well in advance of the time when any tree showed irreversible increases in radius.

In late winter *Larix* exhibited a degree of radial stability that resembled the deciduous dicots more than the evergreen conifers. This supports the hypothesis expressed above that the radial instability which characterizes the latter group is related to their retention of needles and is therefore directly correlated with transpiration.

The grand period of growth of *Larix* began slightly later than the average of each of the two groups. Also, in both summers the shrinkage correlated with drought was more pronounced than that of the average deciduous dicot or evergreen conifer. Thus, the behavior of *Larix* did not consistently resemble that of either deciduous or evergreen trees, except for the matter of radial stability in which it was more like the deciduous dicots.

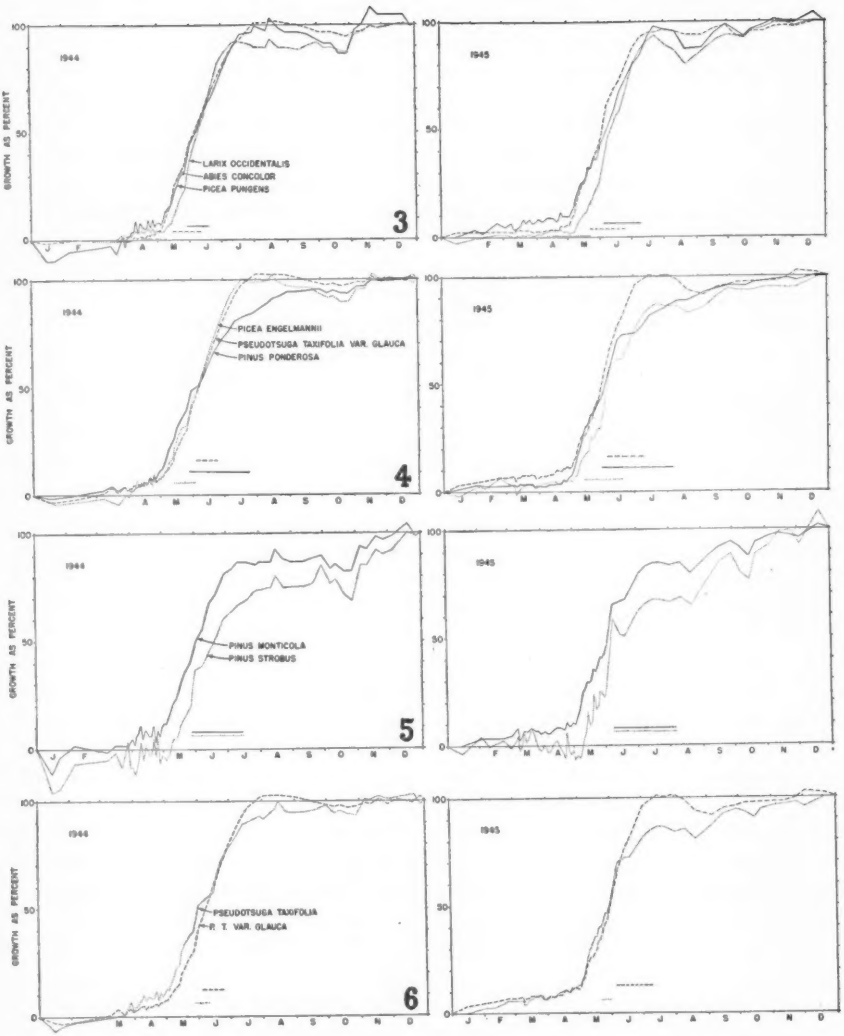
Comparisons among trees native to different altitudes

The inclusion of *Pinus ponderosa*, *Pseudotsuga taxifolia* var. *glauca*, and *Picea engelmannii* in the series of conifers under study made it possible to observe any fundamental adaptations to different climates which may exist among species which grow at different altitudes on the same mountain slope. *Pinus* is native to low altitudes in the Rocky Mountains where the climate is warm and dry. *Picea* occurs at high altitudes where the climate is cool and wet, whereas *Pseudotsuga* occurs at intermediate elevations, overlapping the upper limits of *Pinus* and the lower limits of *Picea*. Obviously, *Pinus* was growing under environmental conditions in the arboretum which most closely approximated its native surroundings, while *Picea* was the farthest from its ecologic optimum.

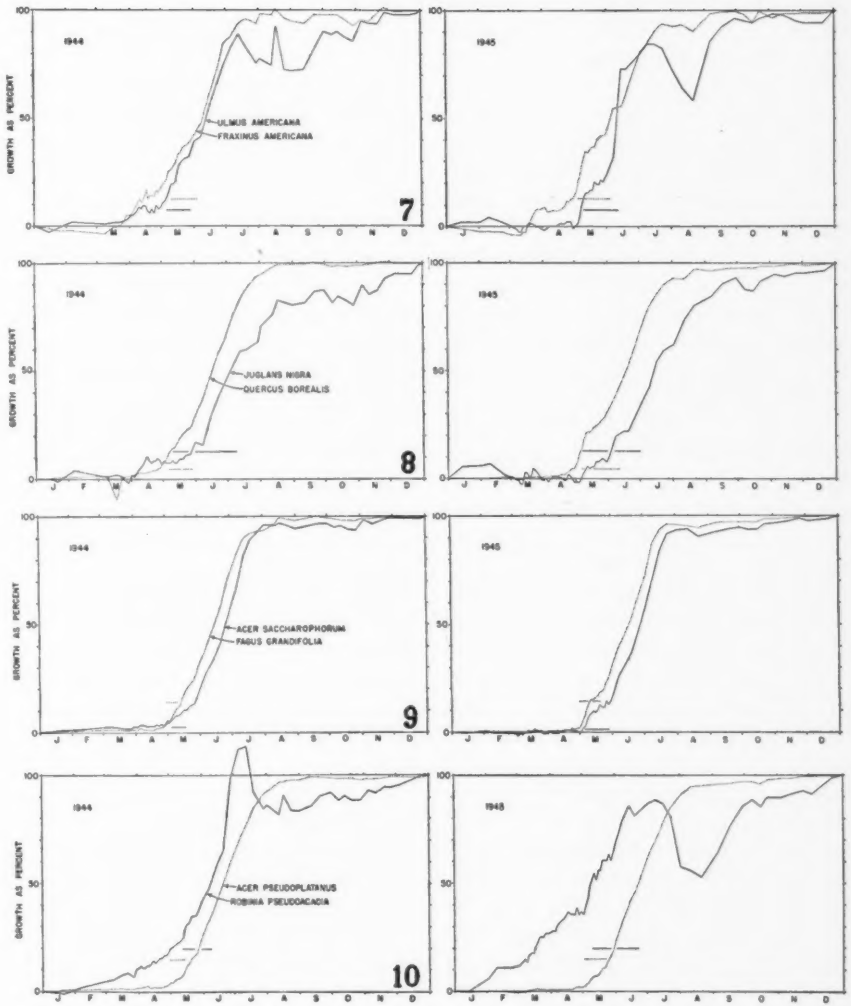
The trunks of *Picea* (fig. 4) were more affected by weather changes from January through April than were those of the other two species, showing pronounced periods of shrinkage and expansion. The radii of *Pinus* fluctuated the least at this season, with *Pseudotsuga* intermediate. Thus the degree of response to variations in winter weather at Moscow was directly correlated with altitudinal distribution.

In 1945 the grand period of growth began slightly earliest in *Pinus* and latest in *Picea*, with *Pseudotsuga* intermediate. This is exactly the reverse of the order which might be expected, assuming that species of cold climates have lower cardinal temperatures than those of warmer regions. However, the data of 1944 show that there is no consistent difference in time of commencement of growth, nor in behavior during the main growing season.

In early June the behavior of *Pinus* diverged considerably from that of the others. The growth rate of this tree was strongly depressed at this time, although rather steady enlargement at an ever slower rate characterized the remainder of the calendar year. *Picea* and *Pseudotsuga*, in contrast, continued rapid growth distinctly later in summer, but then these trees shrank more than did the pine. It is



FIGS. 3–6.—Radial changes in stems in percentage of total yearly increment for species as shown



FIGS. 7–10.—Radial changes in stems in percentage of total yearly increment for species as shown

significant to note that the shrinkage in *Pinus* is essentially autogenous, for, having a much deeper root system than either *Picea* or *Pseudotsuga*, it could not have been affected by the progressive downward desiccation of the soil until after the others had suffered considerably. If the curve for *Pinus* were smoothed, there would be no absolutely quiescent period at any season. This tree at Moscow grows rapidly during May and June, slowly during July, and then continues enlargement at a very slow and somewhat irregular rate throughout autumn and winter until the next grand period of growth begins.

Among other evergreen conifers studied which are native to the Rocky Mountains, *Picea pungens* and *Abies concolor* (fig. 3) have the closest ecologic affinities with *Pseudotsuga*, while *Pinus monticola* (fig. 5) is ecologically the more closely related to *Picea engelmannii*. When the curves of all these trees are compared in relation to altitudinal distribution, it becomes apparent that there are no evident correlations between the native climates and radial behavior at Moscow. The chief possible exception to this may be *Pinus ponderosa* which, in the broader comparison, is outstanding for its early reduction in growth rate and subsequent freedom from shrinkage due to drought.

In the southern Rockies PEARSON (2) has found that apical growth is related to altitudinal distribution in that, whenever two species characteristic of different altitudinal ranges are observed on the same habitat, the one from the higher altitude usually begins growth the earlier. His observations, therefore, indicate successively lower minimal temperatures for apical growth in species from successively higher altitudes. This relationship does not extend to radial growth in the group of trees under study at Moscow. Nor was there any consistent relationship between altitudinal distribution and the period of leaf growth. Cambial activity in 1945 appeared to begin in exactly the reverse of the order that would be expected from PEARSON's observations on apical growth in the southern Rockies!

COMPARISON OF PINUS MONTICOLA AND P. STROBUS

The ranges of *Pinus monticola*, native to mountainous regions of western North America, and *P. strobus*, native to eastern North America, do not overlap. However, these species are very closely related ecologically and taxonomically and appear to have been derived from a common Cenozoic ancestor. Both grow in favorably moist soils, but the western species grows under much the more variable climate with respect to diurnal temperature fluctuations. The two groups of trees studied, three individuals in each, are perfectly matched. The stands are adjacent, the individuals are of approximately equal diameter and height, and the plantations were made with equal spacing.

No other trees exhibited as much relative shrinkage in January, 1944, as did these pines (fig. 5), a behavior which may be largely attributable to the extreme softness of their wood. In both 1944 and 1945 *P. monticola* began rapid growth a few days earlier than did *P. strobus*. The only other notable divergences between the species were observed just prior to the grand period of growth when shrinkage and expansions were occasionally antithetic. In general, *P. strobus* appeared to exhibit the more violent responses to weather variations, but this difference is more apparent than real, for its lower net increment, especially in 1945 (table 1), tends to exaggerate the

degree of response. The most conspicuous feature of the curves is the manner in which they parallel each other throughout most of the year.

Comparison of Pseudotsuga taxifolia and P. t. var. glauca

The pronounced physiologic differences between *Pseudotsuga taxifolia* from the oceanic climatic region along the Pacific Coast and *P. t.* var. *glauca* from the more continental climate of the Rocky Mountain region have long been recognized in silviculture, although taxonomists usually have not considered the morphologic differences sufficient to warrant nomenclatorial recognition. The species along the coast grows chiefly on habitats where drought is slight and transitory, while the inland variety is adapted to a climate that is distinctly drier.

Radial behavior in the species and its variety (fig. 6) differed considerably, but for the most part inconsistently, during the 2 years of study. The chief difference observed both years was that the trees from the coast ceased rapid growth earlier than those from the interior; then subsequently the latter showed a more definite response to cumulative summer drought. In this respect the coastal trees bore approximately the same relation to the interior variety that was noted when *Pinus ponderosa* was compared with the latter. Thus the coastal trees, which, on account of the higher rainfall of their native climate, might be expected to exhibit a behavior more characteristic of a mesophyte, gave responses more nearly resembling a species that is distinctly more xerophytic than the interior variety! It is still possible that this difference in behavior may have resulted from ecotypic specialization, for the exact source of the seed of the two plantations is unknown, and there are a few localities on the Pacific slope that are drier than the wettest areas where *Pseudotsuga* can be found in the interior.

Relationship of wood structure to beginning of radial growth

Several students of tree growth have concluded that trees with ring-porous xylem begin growth earlier than those with diffuse-porous xylem. In the present series of observations the exact time of beginning of radial growth is not easily determined from the radial measurements, but, if it may be assumed that growth among the species began in the same order in which their curves crossed the 10% line, the data obtained at Moscow (table 2) cast considerable doubt as to the fundamental significance of the earlier conclusion.

It is true that some ring-porous species were earlier than some diffuse-porous species, but the dates are almost identical for the ring-porous *Ulmus* (fig. 7) and *Quercus* (fig. 8) and the diffuse-porous *Fagus* (fig. 9). Furthermore, the semi-ring-porous *Juglans* is not intermediate, as would be expected if the hypothesis were significant, but is later than any of the three diffuse-porous species.

When the nonporous conifers are compared with the porous dicots, the 10% point was reached earlier in *Robinia* (fig. 10) and *Fraxinus* (fig. 7), and later in the two species of *Acer* (figs. 9, 10), than the series of dates representing the conifers (table 2).

Period of foliar development

On account of the height and crowded condition of the trees in the arboretum, and because the buds of some trees undergo a long period of gradual swelling, it was not feasible to attempt an accurate

comparison of the beginning of bud activity with that of radial activity. However, records were kept of the period extending from the time when leaves (leaf sheaths of pines) were first visible to their apparent attainment of full size, as viewed from the ground. This period of foliar development is indicated on each graph by horizontal lines of the same types used to designate the seasonal courses of radial change in each species.

Relation of tree growth to frost-free season

In 1944 a temperature of 0° C. or lower was recorded in the louvered box at the weather station as late as May 3 and as early as September 18. In 1945 the last date was June 14 and the earliest August 20. The frost-free seasons were 138 and 67 days long, respectively.

In 1944 the average tree (fig. 2) exhibited a short period of growth in early April and then began its major growing season immediately after the last frost on May 3. A number of species included in this average began to grow well in advance of the frost-free season. Growth in nearly all trees had ceased many weeks before the first frost in the autumn of that year.

In 1945, 50% or more of the season's growth (fig. 2) was completed before the last frost in spring, and growth had been curtailed by drought well before the first frost in autumn of that year. The data thus show no correlation between the

TABLE 2

Comparison of dates of attainment of 10% of net annual increment in relation to types of wood structure

Wood type	Tree	1944	1945	Av.
Ring-porous	Robinia pseudoacacia	Mar 29	Feb 6	Mar 4
	Fraxinus americana	Apr 6	Apr 23	Apr 14
	Quercus borealis	May 4	May 3	May 3
	Ulmus americana	Apr 30	May 9	May 4
Semi-ring-porous	Juglans nigra	May 20	May 31	May 25
Diffuse-porous	Fagus grandifolia	Apr 30	May 7	May 3
	Acer saccharophorum	May 16	May 17	May 16
	A. pseudoplatanus	May 16	May 19	May 17
Nonporous	Pseudotsuga taxifolia	Apr 30	Apr 23	Apr 27
	P. t. var. glauca	May 4	Apr 23	Apr 29
	Pinus ponderosa	Apr 28	May 3	Apr 30
	P. monticola	Apr 28	May 2	Apr 30
	Picea engelmannii	May 4	May 7	May 5
	P. pungens	May 6	May 3	May 5
	Abies concolor	May 8	May 5	May 7
	Larix occidentalis	May 14	May 15	May 14
	Pinus strobus	May 16	May 14	May 15

When the dates of leafing seasons are averaged (fig. 2), it appears that the new needles of the evergreen conifers typically began to emerge as the dicot leaves attained full expansion. The needles of the deciduous conifer *Larix* (fig. 3) developed quite early, at a season more characteristic of the deciduous dicots than of the evergreen conifers.

In both 1944 and 1945 radial growth began slightly later in *Pinus strobus* than in *P. monticola* (fig. 5), but in neither year could any difference in the period of foliar development be detected.

frost-free season and the season of radial growth. In 1944 the grand period of growth was shorter than the frost-free season; in 1945 it was longer.

About half of the trees were clearly affected by the unseasonal drop in temperature which occurred during the middle of the growing season of 1945, on June 14. Responses varying from a slowing of the growth rate to a pronounced shrinkage that was not compensated for several days or more were observed in the evergreen species *Picea engelmannii*, *Pinus ponderosa*, *P. strobus*, and *Pseudotsuga taxifolia*, and in the deciduous species *Fraxinus americana*, *Juglans nigra*, *Robinia pseudoacacia*, and *Ulmus americana*.

Behavior of Robinia pseudoacacia

In some respects the radial behavior of *Robinia pseudoacacia* (fig. 10) was unique. In both summers the grand period of growth terminated very abruptly in early July, following which the tree shrank rapidly, losing about 30% of its total annual increment. Shrinkage in the summer of 1944 was not made up until the following February. In 1943 (data not included in figures) shrinkage did not begin until after the first week in August, and recovery was not completed until after the first week in March, 1944.

A second remarkable feature is the earliness of beginning of the grand period of growth. Rapid enlargement began in early March, 1945, whereas the average date for this event in all species was 2 months later (fig. 2)!

Summary

1. Radial changes throughout the years 1944 and 1945 were studied in seventeen species of trees at Moscow, Idaho, in a region originally covered with prairie. Subsequent to their establishment in an arboretum the trees have not been irrigated, and all have become sufficiently adjusted to the environment to show approximately normal growth.

2. The groups of eight evergreen conifer (*Abies concolor*, *Picea engelmannii*, *P. pungens*, *Pinus monticola*, *P. ponderosa*, *P. strobus*, *Pseudotsuga taxifolia*, and *P. t.* var. *glauca*) and eight deciduous dicots (*Acer pseudoplatanus*, *A. saccharophorum*, *Fagus grandifolia*, *Fraxinus americana*, *Juglans nigra*, *Quercus borealis*, *Robinia pseudoacacia*, and *Ulmus americana*) that were studied began to grow at approximately the same time in both years. During the early part of the grand period of growth the evergreens grew more rapidly than the deciduous dicots, but in the later part of this period the relation was reversed. The evergreens shrank the most in response to drought in summer. Very slow enlargement was observed during autumn and winter in nearly all species, but this was frequently interrupted by periods of shrinkage. In a few instances winter swelling was in part definitely a rehydration of tissues that had suffered marked shrinkage during the preceding summer. In other cases winter swelling probably represented belated hydration of cells produced in summer. The radii of evergreens were less stable during winter than were those of deciduous trees, a phenomenon which is probably related to needle retention, as this habit makes the plants relatively sensitive to transpiration stress in winter.

3. In most respects, radial behavior of the deciduous conifer *Larix occidentalis* did not consistently parallel either that of the other conifers or that of the deciduous dicots, but in its radial stabil-

ity during winter and in its earliness of foliation in spring it resembled the deciduous dicots closely.

4. The inclusion of *Pinus ponderosa*, *Pseudotsuga taxifolia* var. *glauca*, and *Picea engelmannii* in the series of trees provided excellent opportunity to compare the behavior of species native to low, intermediate, and high altitudes in the Rocky Mountains. No consistent differences in behavior were found that could be definitely related to altitudinal distribution. *Pinus ponderosa* exhibited a pronounced autogenous retardation of growth that began early in summer. Subsequently this species did not seem as much affected by the normal summer drought as were the species from higher altitudes, but this behavior appears to be more a peculiarity of the species than a fundamental adaptation to the vertical gradient in climate on mountain slopes.

5. Radial changes in *Pinus monticola* paralleled those of *P. strobus* very closely, except that the former regularly began to grow a few days earlier.

6. *Pseudotsuga taxifolia*, native to the Pacific slope, responded in a manner that indicated a greater degree of xerophytism than possessed by trees representing *P. t.* var. *glauca* of the Rocky Mountain region. The growth of the trees from the coast slowed down earlier in summer, and these trees shrank less in response to late-summer drought. Although there is no record of the exact source of the seed, this difference in behavior may reflect ecotypic specialization, for the driest habitats supporting *Pseudotsuga* along the coast are drier than the wettest habitats in which the species may be found in the interior.

7. No consistent relationship was found between xylem structure and earliness of cambial activity in spring.

8. In general, the leaves of the deciduous trees were nearly fully developed before the new foliage of the evergreens made its appearance.

9. The growing seasons of the trees began before the frost season had ended, and growth stopped well in advance of the first frost in autumn. A drop in temperature to 0° C. on June 14, 1945, at about the middle of the grand period of growth, temporarily produced either a reduction in the rate of growth or shrinkage in about half of the species of evergreens and in half of the species of deciduous trees.

10. *Robinia pseudoacacia* is unique in exhibiting a marked shrinkage, amounting to about 30% of its net annual increment, which took place abruptly after the grand period of growth had ended in both summers. In 1945 this shrinkage was not made up until the following February or March.

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A COMPARISON OF THE EFFECTIVENESS OF 2,4-DICHLOROPHENOXYACETIC ACID AND FOUR OF ITS SALTS IN INDUCING HISTOLOGICAL RESPONSES IN BEAN PLANTS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 586

MARY AILEEN MURRAY AND A. GERALDINE WHITING

Introduction

2,4-Dichlorophenoxyacetic acid (2,4-D) and its various derivatives have been tested in many experiments and have found wide practical application as plant growth-regulating substances. Among the derivatives, several salts of the acid have been used, especially those which are more soluble in water than the acid itself. In early experiments with 2,4-D and with other substituted phenoxy compounds, ZIMMERMAN and HITCHCOCK (11) and ZIMMERMAN (10) reported that the salts, esters, and amides were approximately equal in activity to the acid. Considerable experimentation since the early work has resulted in a modification of this evaluation. HAMNER *et al.* (3) made the general statement that the esters are more effective than the acid and the acid more effective than the salts. They found that the herbicidal action of the sodium salt could be greatly increased when it was applied in an acid solution. MARTH and DAVIS (6) used the ammonium and the sodium salts as well as the acid in herbicidal spray tests on various weeds. They found all three forms effective in causing the death of a number of plants but suggested that certain plants were more sensitive to the acid than to the sodium salt. ENNIS and BOYD (2) reported after extensive spray treatments of many types of broad-leaved plants that the effectiveness of the ammonium salt was not statistically different from that of the acid as a growth-regulating substance. On the other hand, TAYLOR (9) found in general that 2,4-D caused greater inhibition of growth than did the ammonium salt when young wheat plants were grown in nutrient solutions containing either of these compounds.

The present experiment compares the growth-regulating activity of 2,4-D and four of its salts on the basis of histological response induced in the bean plant. The treatment was made on young, decapitated plants in a manner similar to that first used by KRAUS *et al.* (5) and later extended to a series of experiments testing various growth-regulating substances, including 2,4-D and related phenoxy compounds recently reported by BEAL (1). Plants of *Phaseolus vulgaris* var. Red Kidney were grown in flats in the greenhouse. After selection for uniformity and vigor of growth the plants were divided into five lots. Each lot received treatment with one of the following compounds mixed in 0.5% concentration by weight in lanolin: 2,4-dichlorophenoxyacetic acid (2,4-D), ammonium 2,4-dichlorophenoxyacetate, copper 2,4-dichlorophenoxyacetate, calcium 2,4-dichlorophenoxyacetate, and magnesium 2,4-dichlorophenoxyacetate. The plants were decapitated in the upper part of the second internode, and the lanolin paste was applied to the cut surface. The decapitated stems were collected at given intervals up to 30 days, killed and fixed in Navashin's fluid, imbedded by the

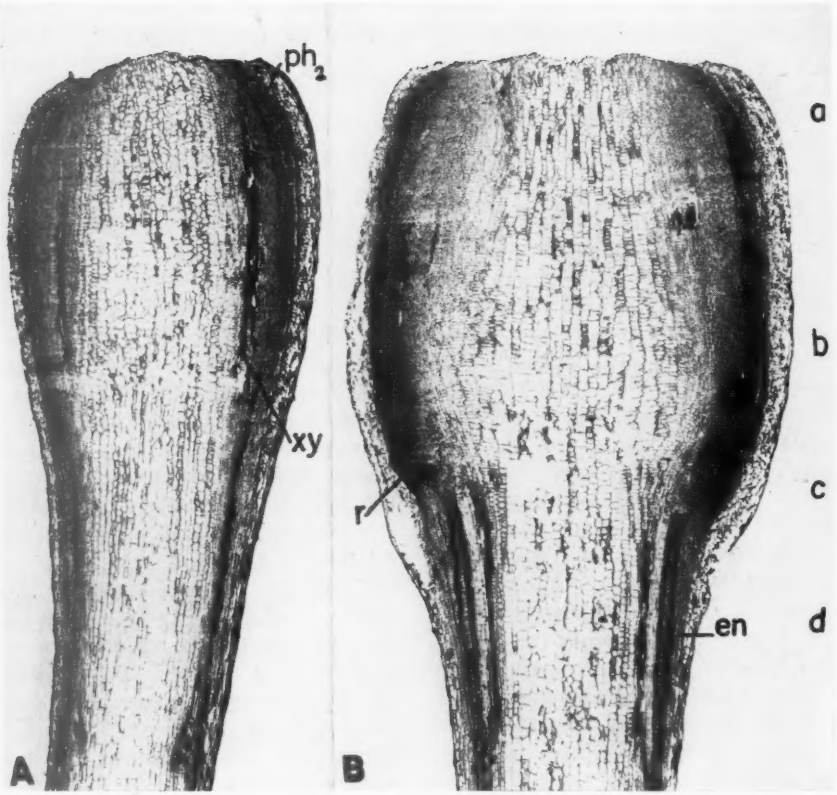

FIG. 1.—Longisections of decapitated second internodes treated with 0.5% 2,4-D. Young stages in enlargement of stem tips to form apical tumors. *A*, 3 days after treatment: *ph*$_2$, secondary phloem; *xy*, secondary xylem. Parenchymatous tissues from inner cortical parenchyma to pith proliferating. Pith inactive. *B*, 5 days. Further proliferation of parenchymatous tissues and enlargement of tumor; greatest vertical depth of response in endodermis (*en*); initiation of root primordium (*r*). On basis of meristematic activity (generally indicated by density of area) stem tip may be divided into zones: at upper levels, zone of limited proliferation (*a*); at intermediate levels, zone of major proliferation (*b*); at shoulder of tumor, root zone (*c*); at lower levels of response, zone of minor proliferation (*d*).

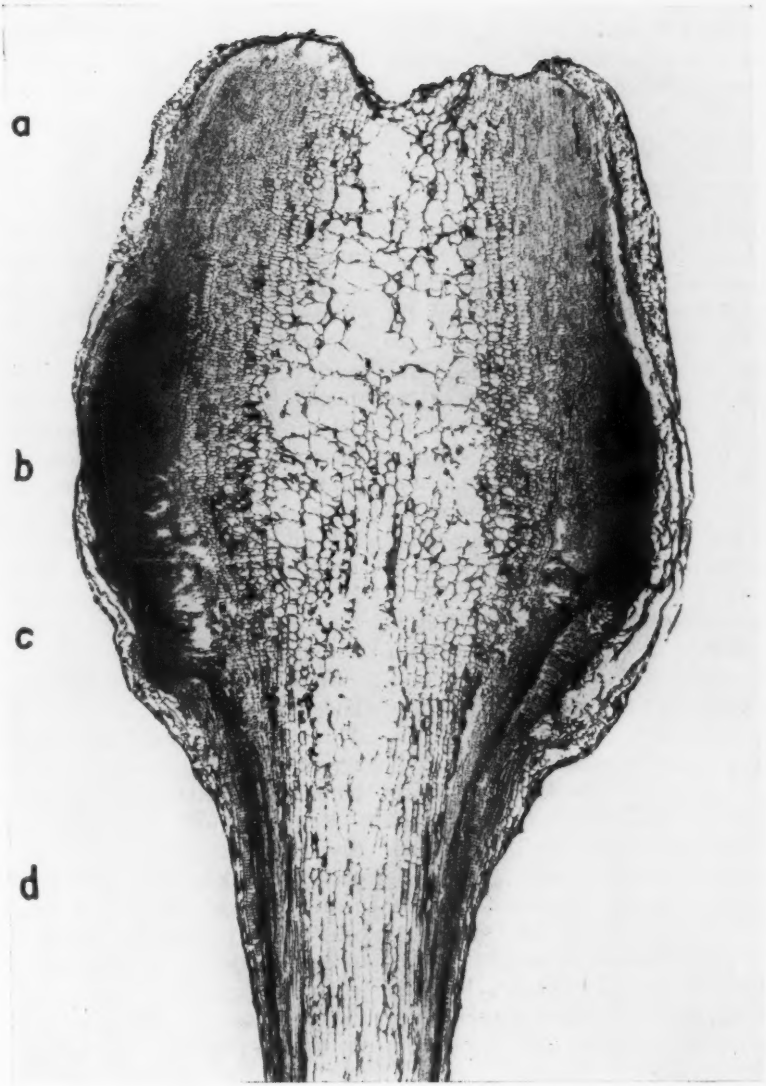

FIG. 2.—Longisection, 12 days after treatment with 2,4-D. Zones *a–d* same as in fig. 1*B*. Mature apical tumor showing zonation in pattern of proliferation and maturation of tissues. Zone *a*, proliferated tissues matured as parenchyma; many dying cells at cut surface, in pith, and from pericycle outward. Zone *b*, derivatives of proliferated tissues from primary phloem inward to pith actively meristematic; outer tissues generally collapsed. Zone *c*, tissues forming root primordia remaining meristematic; other proliferated tissues maturing as parenchyma or as reticulate tracheids. Zone *d*, previously active cells maturing as parenchyma and as scattered reticulate tracheids; occasionally vascular bundles formed, especially in phloem.

butyl alcohol–paraffin method, sectioned at 12–15 μ, and stained with a modified Flemming's triple stain.

Observations

Early responses

Response in decapitated stems was initiated during the first 24 hours. Changes in the appearance of these stem tips, almost imperceptible during the first day, were distinct by the end of the second day. The green color faded, the originally inconspicuous ribbing of the stem became more marked, and the tip began to enlarge in the formation of a tumor. All treatments resulted in a similar series of early reactions. During the third and fourth days the tumors grew considerably in size and began to show characteristic differences. The depth or downward extent of response in the stem was greatest in those tips treated with 2,4-D and with the ammonium salt, somewhat less when the copper salt was used, and distinctly shallower with the calcium and magnesium salts (figs. 1*A*, 3). The shapes of the tumors likewise varied significantly. The deeper tumors resulting from treatment with 2,4-D and with the ammonium salt were club-shaped and slightly constricted at the top; the tumor induced by the copper salt was intermediate in position, somewhat variable in shape, but often bulbous at the upper end; the shallow tumors induced by the calcium and magnesium salts were flared at the cut surface and gradually tapered below. With all treatments the tumor was greenish-yellow in color, waxy on the lateral surface, and rather dense and firm in texture by the fourth day.

Histological examination very clearly showed the tissue proliferations underlying the changes seen in gross responses. For all five substances there was fundamental similarity in the tissues which responded and in the type of response induced. Certain variations became increasingly marked, however, with the continued development of the tumors. In the following analysis of early response, development by tissues is traced through the first 4 days after treatment.

The epidermis exhibited no specific response to 2,4-D or to its salts. The outer cortical parenchyma showed little early response aside from cell enlargement. This occurred for several millimeters downward in the stem, extending generally over those portions showing marked increase in diameter. In some cases cell enlargement was followed by cell division. This was a frequent and characteristic response to the magnesium salt (fig. 8*A*), although it might occur in the cortex over especially active bundles with any of the other substances. Cell division was in various planes, any one cell undergoing only one or two divisions. This response followed the initiation of activity in tissues centrad to it and was not marked until the third or fourth day.

Response in the inner cortical parenchyma was similar to that of the outer cortical parenchyma but was earlier and greater in degree. By the end of 24 hours, cells adjacent to the endodermis showed increase in density of cytoplasm and in size of nuclei. In those stems treated with the magnesium or calcium salt, divisions in various planes occurred during the first day after treatment, and in stems treated with the ammonium salt and 2,4-D during the second day. As proliferation continued, this tissue became indistinguishable from the endodermis.

The endodermis was the first tissue to respond and showed proliferation within approximately 24 hours. Slight cell enlargement (not the radial elongation

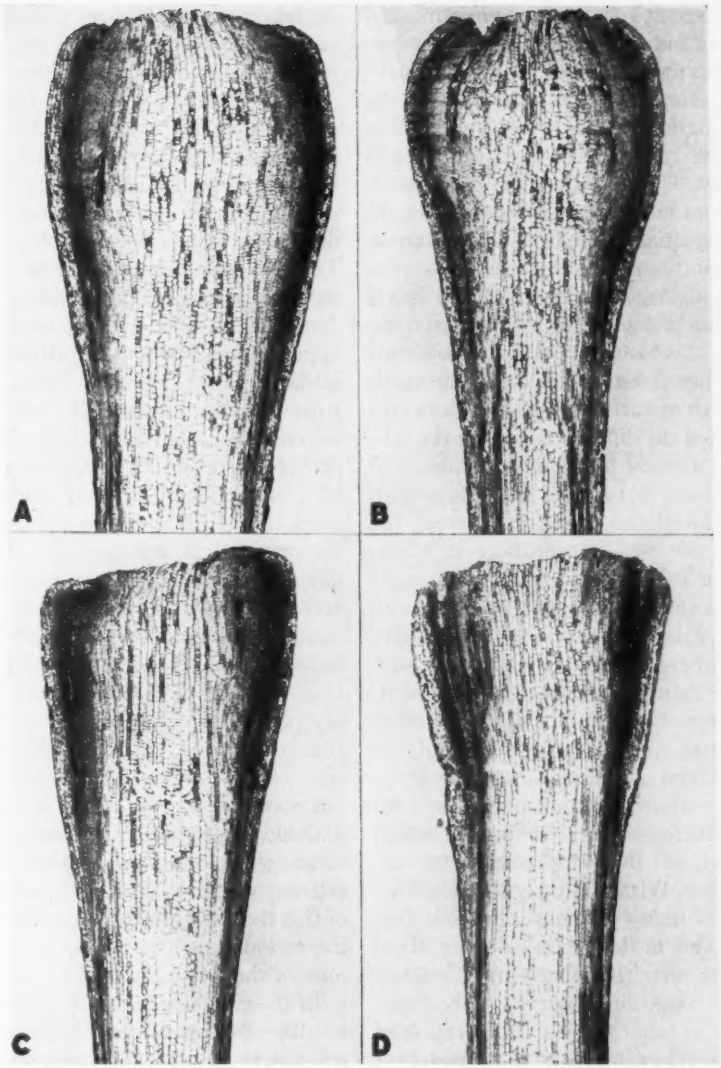

FIG. 3.—Longisections of stem tips 3 days after treatment with 0.5% ammonium (*A*), copper (*B*), calcium (*C*), and magnesium (*D*) salts of 2,4-dichlorophenoxyacetic acid. Tumors arranged in series from deep, club-shaped tumor induced by ammonium salt to shallow, flared tumor induced by magnesium salt. Initial proliferation of parenchymatous tissues at upper levels well advanced; central pith inactive. Note activity in outer cortical parenchyma of two shallower tumors, *C* and *D*. Compare with fig. 1*A*.

characteristic for some growth substances) and conspicuous increase in density of cytoplasm preceded tangential division. The magnesium salt induced the most marked response during the first day, the calcium salt next, and 2,4-D the least. During the next few days divisions continued, in the radial as well as in the tangential plane. Generally a narrow band of meristematic tissue was produced (fig. 14). Except at lower levels where only the endodermis showed response, this band merged with proliferating tissues on either side of it. The vertical depth of this response at 3 days corroborated the differences among the substances already noted in descriptions of the tumors: 2,4-D and the ammonium salt induced the deepest responses, the copper salt was intermediate in effect, and the calcium and magnesium salts induced only shallow reactions (table 1).

The pericycle is generally less sensitive to treatment with growth-regulating substances than tissues on either side of it. The degree of proliferation described here, and most clearly discernible in longisections, is unusual. At upper levels young parenchymatous pericyclic cells slowly increased in size and the nuclei enlarged, but the cytoplasm did not appear dense. Within 2–3 days divisions occurred in many of these large cells (fig. 14*A*). The nuclei resulting from these divisions were frequently multinucleolate, a condition observed only occasionally in other tissues. Proliferation of the pericycle sometimes continued to a point where the cells became indistinguishable from other proliferated tissues. However, the pericycle over the vascular bundles generally remained a conspicuous patch of large, thin-walled cells (fig. 8*A*) which tended to collapse. These cells might collapse as early as 4 days with the copper, calcium, and magnesium salts. At levels where the pericyclic cells had begun to mature as fibers, response by proliferation did not occur.

Proliferation of the parenchymatous cells of both the primary and the secondary phloem was of major importance in the formation of the tumors. Response in the phloem parenchyma was initiated during the first 24 hours after treatment. The cells gradually became more meristematic in appearance, the cytoplasm denser, and the nuclei larger. Some cells appeared enlarged. Cell division was initiated earliest in those stems treated with the magnesium salt, but by the second day this tissue had begun to proliferate in all stems. Divisions were in all planes, frequently four to five divisions taking place before the outline of the original cell was lost. By the third day at levels of greatest meristematic activity the phloem had formed a wide band of proliferated tissue which merged indistinguishably with adjoining areas. However, the sieve tubes of the secondary phloem remained conspicuously unchanged among the meristematic cells (fig. 1*A*). At lower levels in the tumor, meristematic activity in the phloem was limited, often not exceeding the first stages where the outline of the original cell was evident. The depth of response of this tissue was next greatest to that of the endodermis and reached within 2–4 mm. of the latter (fig. 14*B*).

In the cambium, initiation of activity resulting from treatment by the different substances was difficult to determine. At the end of 48 hours the zone of undifferentiated cambial derivatives had widened considerably, and activity had extended across many of the rays. Activity continued through the third and fourth days. In stems treated with the magnesium salt, maturation of xylem tracheids among the inner derivatives of the

cambium commenced by the second day at 0.5 mm. and extended downward. Similar maturation was noted with the other substances by 3 days, at 1 mm. with the calcium salt, at 3 mm. with the copper salt, and at 4 mm. with both 2,4-D and the ammonium salt.

Parenchyma in that portion of the ray lying outside the interfascicular cambium responded in the upper levels in a manner similar to the phloem parenchyma. Together these formed a broad band of small-celled, meristematic tissues. By the fourth day at upper levels in the shallow tumors and at middle levels in the deeper tumors the meristematic activity of the rays exceeded that of the phloem. This radial pattern of alternating patches of intensely active rays and less active bundles was the first indication of developing root primordia.

The inner ray, that portion extending from the cambial derivatives to the protoxylem points, was markedly less active. During the first day the cells increased in size slightly, and the nuclei became particularly conspicuous. Cell divisions were observed within 24 hours after treatment with the magnesium and calcium salts and within 48 hours with the other substances. Several cell divisions might occur before the original cell outline was lost. The xylem parenchyma attained a similar degree of activity. In many cases inactive lignified primary xylem elements were widely separated by the enlargement or proliferation of intervening parenchymatous cells. Gradually this activity extended inward to the pith parenchyma centrad to the protoxylem points, especially in tumors induced by the magnesium salt. However, the response of this inner portion of the stem was never so great as that in the outer portion. The earliest maturation of proliferated cells occurred among the derivatives of proliferation in the ray and xylem parenchyma. Reticulate tracheids were observed as early as the third day in tumors induced by magnesium and calcium salts, but not until several days later in stems treated with the other substances. The central pith was inactive.

TABLE 1

DEPTH OF RESPONSE IN ENDODERMIS IN TUMORS INDUCED BY 2,4-DICHLOROPHENOXYACETIC ACID AND FOUR OF ITS SALTS, ON VARIOUS DAYS AFTER TREATMENT

SUBSTANCE	DEPTH OF RESPONSE (MM.)						
	2 days	3 days	4 days	5 days	7 days	12 days	20 days
2,4-D	10.3	11.6	15.0	13.5	13.0	12.6	11.5
NH_4 salt	9.5	12.0	14.0	13.0	14.3	13.0	11.0
Cu salt	8.0	11.6	14.0	12.6	12.0	11.3	11.6
Ca salt	6.5	10.0	11.0	11.0	10.0	10.0	12.3
Mg salt	6.0	9.3	9.0	10.0	9.0	9.5	8.3

LATER RESPONSES

ZONATION.—By the fifth day the pattern of response in the various tissues was fully established and the structure of the tumor could be studied as a whole. In the early response it was apparent that the degree and character of proliferation varied at different levels. Analysis of these differences disclosed a pattern of zonation. The pattern, as most fully de-

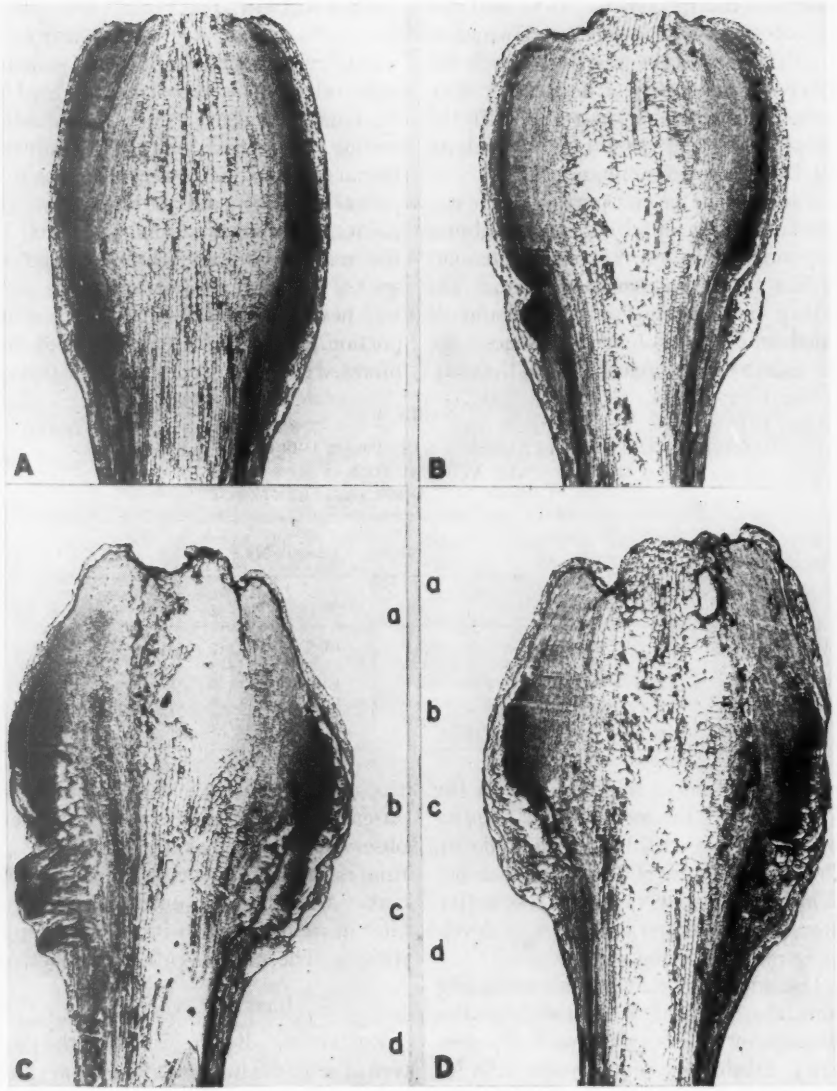

FIG. 4.—*Above*, 5 days; *below*, 12 days after treatment. *Left*, ammonium salt; *right*, copper salt of 2,4-D. Zones *a–d* same as in fig. 1*B*. Zone of limited proliferation evident in deep tumors induced by these salts. In root zone (*c*) note upper fasciated or multiple root primordium and lower single primordium in *C*.

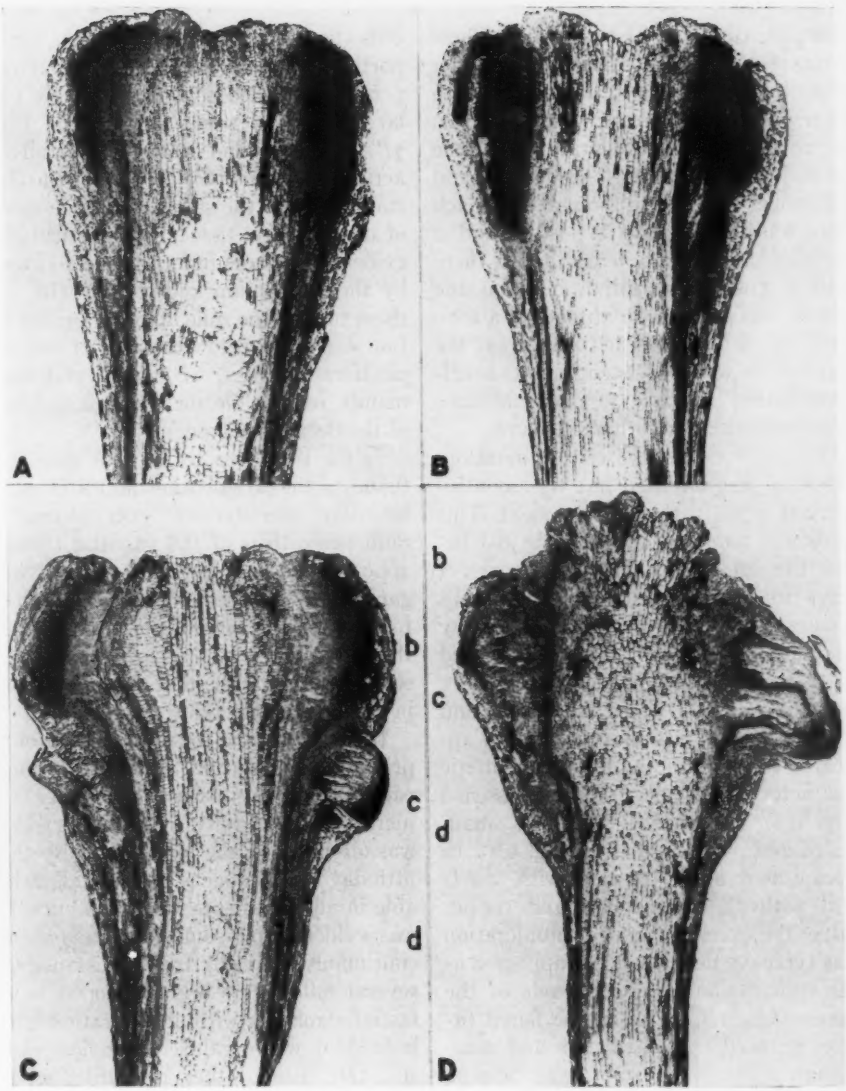

FIG. 5.—*Above*, 5 days; *below*, 12 days after treatment. *Left*, calcium salt; *right*, magnesium salt of 2,4-D. Zones *b–d* same as in fig. 1*B*. Zone of limited proliferation lacking for shallow tumors induced by these salts. Proliferation above original level of cut surface characteristic response to magnesium salt. Figures 4 and 5 show variation in form of tumors resulting from treatment with different salts of 2,4-D and are arranged to indicate decreases in vertical depth of response with accompanying changes in zonation. (See also table 2). Characteristic levels of root zone (*c*) are shown: lowest with ammonium salt (fig. 4*C*) and nearly at cut surface with magnesium salt (fig. 5*D*). Figs. 3, 4, 5 same magnification.

veloped, consisted of four zones. These zones (figs. 1*B*, 2), arranged in order from the cut surface to the lowest depth of response in the tumor, were as follows: (*a*) zone of limited proliferation; (*b*) zone of major proliferation; (*c*) root zone; and (*d*) zone of minor proliferation. Each zone was distinctive in its position and in its histological character, although there was a gradual transition between the zones. The presence or absence of a zone and the distance of each zone from the cut surface were consistent factors in differentiating response to one substance from response to another substance.

In the zone of limited proliferation, response was initiated but was soon inhibited in its further development. This zone was found at upper levels just below the cut surface. During the first 3 days after treatment parenchymatous tissues from the cortex to the inner ray and about the xylem points proliferated in a manner already described under early response. During the fourth and fifth days this rate of proliferation appeared to diminish and the meristematic character of the cells gradually lessened (fig. 6*A*). Derivatives remained small-celled and parenchymatous (fig. 6*B*). In decapitated stems treated with 2,4-D and with the ammonium and copper salts, the zone of limited proliferation was typically developed and appeared as the constriction at upper levels of the tumor (figs. 1*B*, 2, 4). In the flared tumors induced by the calcium and magnesium salts this zone was absent (fig. 5).

In the zone of major proliferation the greatest and most prolonged meristematic activity occurred. Derivatives of cortical parenchyma, endodermis, phloem parenchyma, cambium, and the outer portion of the ray possessed the dense cytoplasm and large nuclei of dividing cells (fig. 7*A*). Parenchyma in the inner portion of the ray and about the xylem points responded with considerable enlargement and some proliferation (fig. 7*B*). A broad and continuous band of active tissue was formed around the circumference of the stem. This wide band of meristematic tissues was particularly evident in tumors induced by 2,4-D and by the ammonium and copper salts. In these tumors the zone of major proliferation was found below the zone of limited proliferation (figs. 1*B*, 2, 4) and was mainly responsible for the enlargement of the decapitated stem.

In the transition to the root zone the tissues of the rays became more and more intensely meristematic. On alternate radii derivatives of the vascular tissues appeared less active. This type of organization was characteristic of response to the calcium and magnesium salts at the uppermost levels (fig. 9*B*) and represented the greatest extent of proliferation in these flared tumors (fig. 5).

In the root zone the organization of a primordium occurred generally within each ray. The increased intensity of meristematic activity in the outer rays was observed by the fourth day. By the fifth day root histogens were distinguishable in all treatments. In longisection it was evident that some primordia were continuous vertically over a distance of several millimeters and developed in a fasciated manner with no separation into individual, well-organized root tips (figs. 4*C*, 5*D*). From other primordia well-developed individual roots were formed (fig. 4*C*). With all five substances the root zone was unmistakably expressed in protuberances forming an abrupt ridge or shoulder at the base of that portion of the tumor showing the greatest enlargement. Although the distance of the root zone from the surface of application dif-

fered markedly in the responses induced by 2,4-D and its salts, the pattern of proliferation within the stems varied but little (figs. 10, 11). In the vascular bundles alternating with the root primordia, proliferation was relatively slight and the pattern similar to that in the zone of minor proliferation.

The zone of minor proliferation was found at levels where response progressively diminished and finally terminated. Although the total proliferation for this portion of the stem was relatively slight, activity in large bundles was conspicuous, especially when the proliferation was followed by a complex pattern of maturation (fig. 12). In the upper part of this zone considerable proliferation of the endodermis occurred over the large bundles (fig. 12*B*). In stem tips treated with the magnesium salt this proliferation frequently extended through the cortical parenchyma to the epidermis. Similar activity was observed in tumors induced by the calcium salt (fig. 15) but was uncommon and much less marked with the other substances. In all treatments a narrow band of proliferated endodermis was formed over the smaller bundles (fig. 12). At the lowest levels endodermal response decreased to a single division and finally to slight cell enlargement. Pericyclic cells (fig. 15) were often matured as fibers; sometimes the cells were enlarged and proliferated. The greatest proliferation in this zone was observed in the phloem parenchyma, both primary and secondary. Activity in this tissue was marked to a depth almost as great as that in the endodermis (fig. 14*B*). Only traces of response were evident in the cambial region, in the xylem parenchyma, and in the tissues of the ray. Limitation of proliferation principally to the vascular bundles was characteristic of response in the zone of minor proliferation. With all treatments, general maturation of proliferated tissues was found earlier in this zone than in the other zones. Many of the characteristics of the mature condition were evident by the fifth day, especially in those tumors resulting from treatment with the calcium and magnesium salts.

MATURATION.—The greatest rate of enlargement of the tumor occurred during the first week after treatment. Growth thereafter was slower but continued for several weeks more. This growth took place mainly in the zone of major proliferation and in the root zone. The time of maturation and death of the tissues varied in the different tumors. In tumors induced by 2,4-D and by the ammonium and copper salts collapse was evident in the zone of limited proliferation 2 weeks after treatment. Constriction at the tip increased greatly, the tissues changed in color from waxy yellow to brown, and necrosis occurred. By 30 days collapse and death had extended to the lowest levels of response. Decapitated stems treated with the calcium and magnesium salts were firm and green 3 weeks after treatment. By 30 days some collapse and dying were evident. Tissues proliferating above the cut surface in those tumors induced by the magnesium salt remained succulent and active to the end of the period of observation. This was the most prolonged stimulation of tissues noted in this experiment.

In the zone of limited proliferation activity was much reduced by the fifth day. In the small-celled derivatives the cytoplasm was less dense and the nuclei diminished in size. Further development seemed to be inhibited, and the tissues remained parenchymatous for some time (fig. 6*B*). At the end of 12 days the beginning of collapse and death was evident. Commencing at the cut surface and

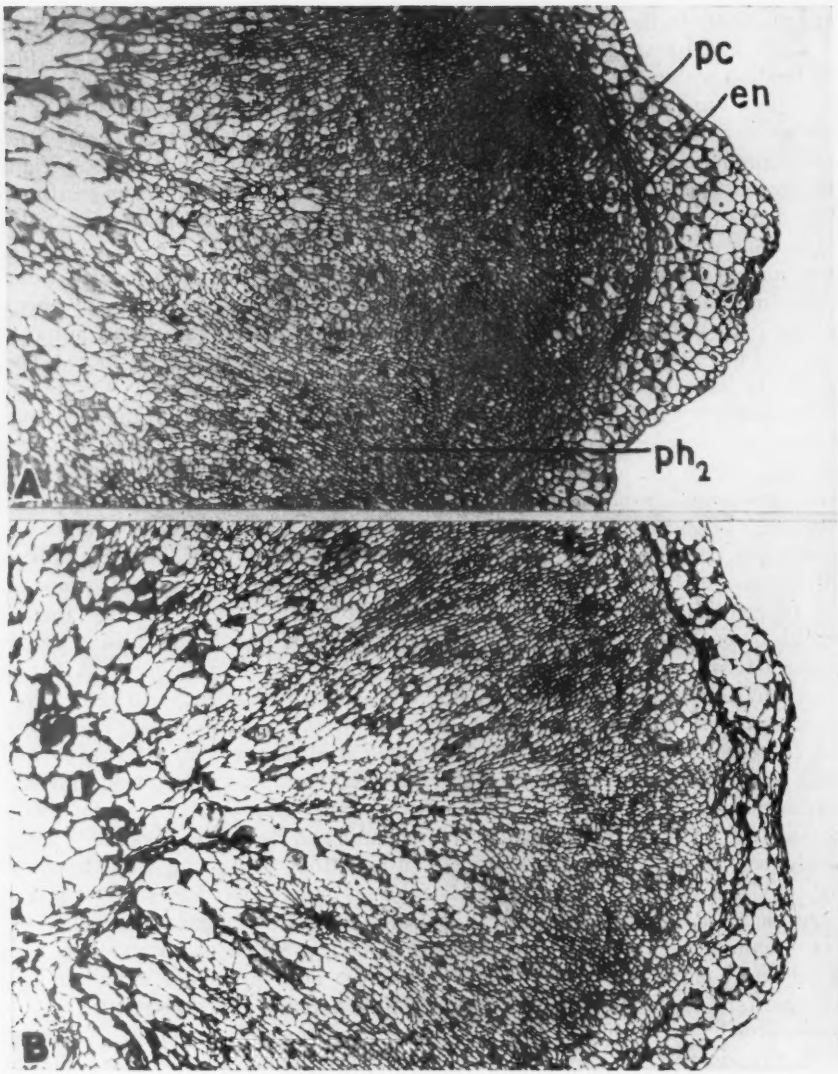

FIG. 6.—Transections of stem tips treated with ammonium salt of 2,4-D; in zone of limited proliferation. *A*, 5 days after treatment, 0.2 mm. below cut surface. Inner cortical parenchyma and endodermis (*en*) slightly proliferated, maturing as parenchyma; pericyclic cells (*pc*) much enlarged and some divided; derivatives of proliferated tissues from primary phloem to pith losing meristematic character. *B*, 12 days after treatment, 1 mm. below cut surface. Response limited to degree of proliferation reached by 5 days. Some tissues collapsing; others matured as parenchyma. Compare with fig. 4*C*.

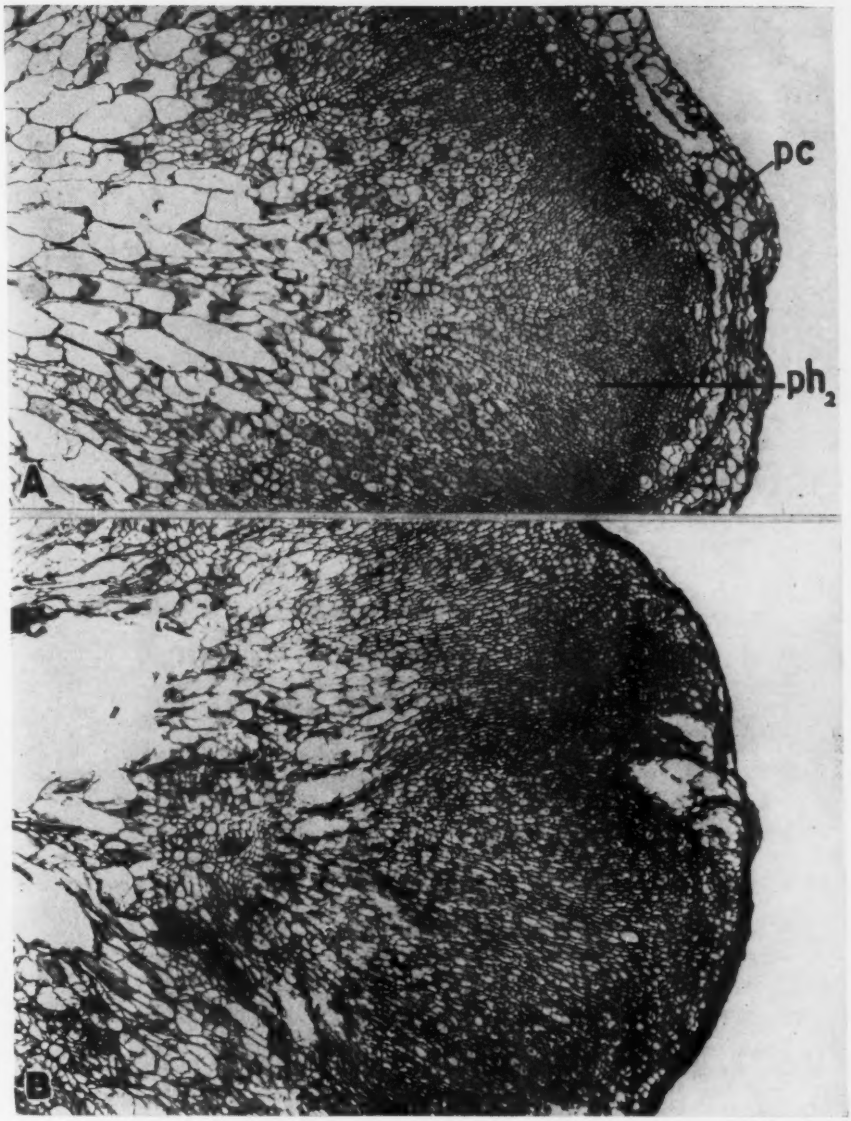

FIG. 7.—Transections of stem tips treated with ammonium salt of 2,4-D; in zone of major proliferation. *A*, same stem as fig. 6*A*, 3 mm. below cut surface: ph_2, secondary phloem. Pericyclic cells much enlarged (*pc*), some collapsed. Parenchymatous tissues from primary phloem to pith strongly meristematic and proliferating; outer portion of rays similarly active. Slight proliferation in xylem parenchyma and in inner portion of rays. Note density of cytoplasm in proliferating cells as compared with fig. 6*A*. *B*, same stem as fig. 6*B*, 4 mm. below cut surface. Collapsed epidermis, cortical parenchyma, endodermis, and pericycle forming heavy outer layer of tumor. Proliferated tissues (phloem, outer portion of ray, and cambial derivatives) generally maturing as parenchyma; some xylem tracheids differentiated. Proliferated cells in xylem parenchyma and in inner portion of ray maturing as reticulate tracheids or remaining meristematic to form vascular bundles.

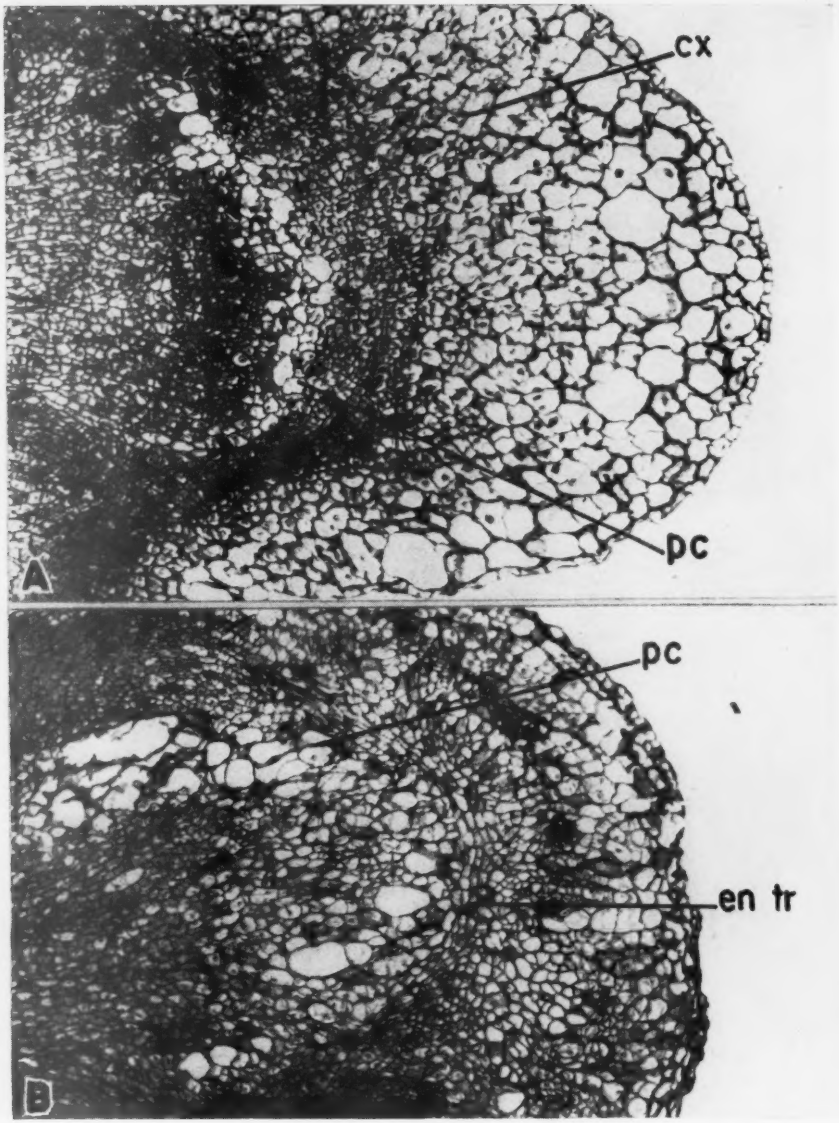

FIG. 8.—Transections of stem tips treated with magnesium salt of 2,4-D. *A*, 4 days after treatment, 1 mm. below cut surface. Greatest proliferation with magnesium salt found at uppermost levels and at this age. Response similar to that noted with ammonium salt at base of zone of major proliferation (5–6 mm.) at 5 days; differences in degree of proliferation between vascular bundles and rays result in scalloped band of active tissues. Note strong proliferation of cortical parenchyma (*cx*) and endodermis, frequent response to magnesium salt. *B*, 5 days after treatment, 0.3 mm. below cut surface. Maturation of proliferated tissues much earlier than that observed in treatment with ammonium salt. (Compare with fig. 7*A*.) Derivatives of proliferated cortical parenchyma, endodermis (*en tr*), and phloem parenchyma maturing as reticulate tracheids or as parenchyma. Some pericyclic cells much enlarged (*pc*), a few collapsed.

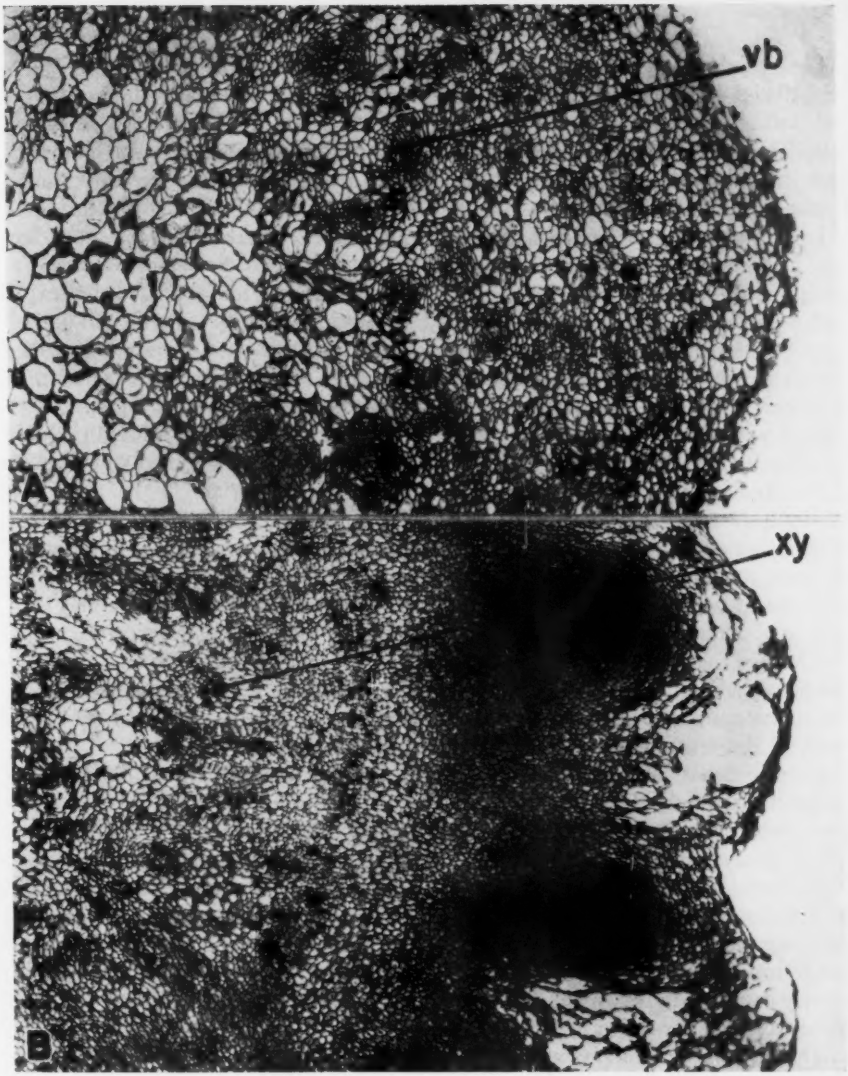

FIG. 9.—Transections of stem tip, 12 days after treatment with magnesium salt of 2,4-D. *A*, 0.5 mm. above original cut surface. Development above original cut surface characteristic of response to magnesium salt. Tumor at this level consists of parenchyma and complex system of small anastomosing vascular bundles (*vb*) and is derived mostly from proliferation of inner rays, xylem, and pith parenchyma. *B*, same stem as *A*, just below cut surface: *xy*, xylem. Marked proliferation in xylem parenchyma, inner portion of ray, and pith; derivatives maturing as parenchyma, as scattered reticulate tracheids, or as small vascular bundles. Upward proliferation of these inner tissues forms tumor above original cut surface. Differences in degree of proliferation in rays and vascular bundles more pronounced than in fig. 8*A*. Many derivatives of proliferation in vascular bundles have collapsed. Highly meristematic character of rays indicates transition to root zone. Outer tissues generally collapsed.

in the endodermis, changes in cytoplasm and nuclei of proliferated tissues preceded disorganization and collapse of the cells. This collapse progressed downward and inward into deeper layers of the tumor (figs. 2, 4*C*, *D*).

In the zone of major proliferation general maturation was longest delayed in decapitated stems treated with 2,4-D and with the ammonium, calcium, and copper salts. In these tumors the outer tissues from the epidermis to the pericycle generally collapsed to form a heavy outer layer. The wide band of proliferated tissues derived from phloem parenchyma and the outer portion of the ray continued activity as long as 2 weeks. Maturation was mainly as parenchyma. In tumors induced by the magnesium salt, maturation was established as early as 5 days. Differentiation of tracheids in proliferated endodermis and phloem parenchyma (fig. 8*B*) emphasized the similarity and proximity of the upper levels of these tumors to the root zone. The inner derivatives of the cambium matured as tracheids, although these were shorter and smaller than the usual xylem tracheids. An exception to generally retarded maturation was found in the proliferated parenchyma of the xylem and inner portion of the ray. Differentiation of reticulate tracheids was observed as early as 3 days in treatment with magnesium and calcium salts and 4 days with 2,4-D and the ammonium and copper salts. Other derivatives matured as parenchyma or remained meristematic and formed a complex pattern of vascular strands. Vascularization was noted with all treatments, but particularly with the copper, calcium, and magnesium salts. This type of maturation in the inner tissues extended downward through the two lower zones, gradually diminishing in extent with decreasing effectiveness of the substance. In decapitated stems treated with the magnesium salt, and sometimes in those treated with the calcium salt, proliferation of these inner tissues at the cut surface accounted for the formation of a portion of the tumor above the original level of the cut surface. With continued development of the tumor this activity extended inward to portions of the pith (figs. 9*A*, *B*) and upward into the distinctive overgrowth at the apex of the tumor induced with the magnesium salt. In other treatments the presence of the zone of limited proliferation effectively prevented development above the cut surface.

In the root zone well-developed root tips remained meristematic until general collapse of the tips occurred. Elongation of roots was relatively slight and in no instance did the tips penetrate through the outermost tissues (fig. 11). Differentiation of the other proliferated tissues was similar to that found in the lowest zone (fig. 11*A*).

Relatively small areas in the zone of minor proliferation exhibited marked activity. The pattern of maturation in these areas, however, was more complex than in any other portion of the stem (fig. 15). Derivatives of endodermis and cortical parenchyma generally matured as parenchyma. Over some large bundles, particularly with the calcium and magnesium salts, reticulate tracheids, fiber-like cells, meristematic patches, and vascular bundles, as well as parenchyma, were noted (figs. 13*B*, 15). The reticulate tracheids in the endodermis connected through the interfascicular rays with the xylem. Maturation in the phloem resulted in large areas of reticulate tracheids intermingled with some parenchyma, scattered vascular bundles, and patches of fiber-like cells. These tracheids connected through the phloem rays with the

xylem. Frequently a band of primary phloem cells adjacent to the pericycle remained meristematic. This was particularly evident with 2,4-D and the ammonium salt but much less so with the magnesium salt (fig. 13). This complex of tissues followed no regular pattern but extended from the endodermis through the pericycle and phloem parenchyma to the cambium (fig. 15) and reached its most elaborate development at the level just below the root zone. The same pattern extended upward through the root zone to the lowest levels of the zone of major proliferation and downward with increasing simplicity to levels where apparent response ceased altogether.

Discussion

In this experiment histological responses induced in bean stems by 2,4-D and by its ammonium, copper, calcium, and magnesium salts were generally similar to those described by BEAL (1) and by SWANSON (8) for 2,4-D. SWANSON considered that each growth-regulating substance induced a pattern of reactions characteristic for that substance. He referred to this as individuality of effect. Despite certain variations in the reactions of bean plants to 2,4-D and to its salts, the responses resulting from any one treatment appeared to fall within the range of effects distinctive for 2,4-D. The differences among the tumors induced by the various substances were observed both in the histological responses and in the zonation. However, it is significant that no response induced by one substance was entirely absent in the reaction to another substance. Where differences existed there was a gradual increase or decrease in this response from one substance to the next. In general, the compounds fell into a series in the following order: at one end 2,4-D and the ammonium salt, in an intermediate position the copper salt, and at the other end the calcium and magnesium salts.

Histological responses to the various substances differed in the following ways: (*a*) rate at which response was initiated and progressed to maturation; (*b*) degree of proliferation in various tissues and at different levels, both of which considerably influenced the shape of the tumor; and (*c*) the extent of maturation, determined by the number of reticulate tracheids and the amount of vascularization in proliferated tissues. Differences within the tissues may be summarized as follows:

1. Proliferation of the outer cortical parenchyma was a frequent response to the magnesium salt, although it might occur to a lesser degree over especially active bundles with any of the other substances.

2. Response in the endodermis was initiated first with the magnesium salt, next with the calcium salt, and last with 2,4-D. A similar gradation was noted in the depth of response in the endodermis (table 1). Likewise, reticulate tracheids and small vascular bundles were a common form of maturation over large bundles with the magnesium salt, although they were found to a lesser degree and more infrequently with the other substances (figs. 12, 13).

3. Divisions were observed in the inner portion of the ray and in the xylem parenchyma within 24 hours with the magnesium and calcium salts but not until 48 hours with the other substances. Maturation of derivatives as reticulate tracheids was noted on the third day with the magnesium and calcium salts but not until the fifth day with the other substances.

4. Pith parenchyma centrad to the protoxylem points was particularly active with the magnesium salt, somewhat

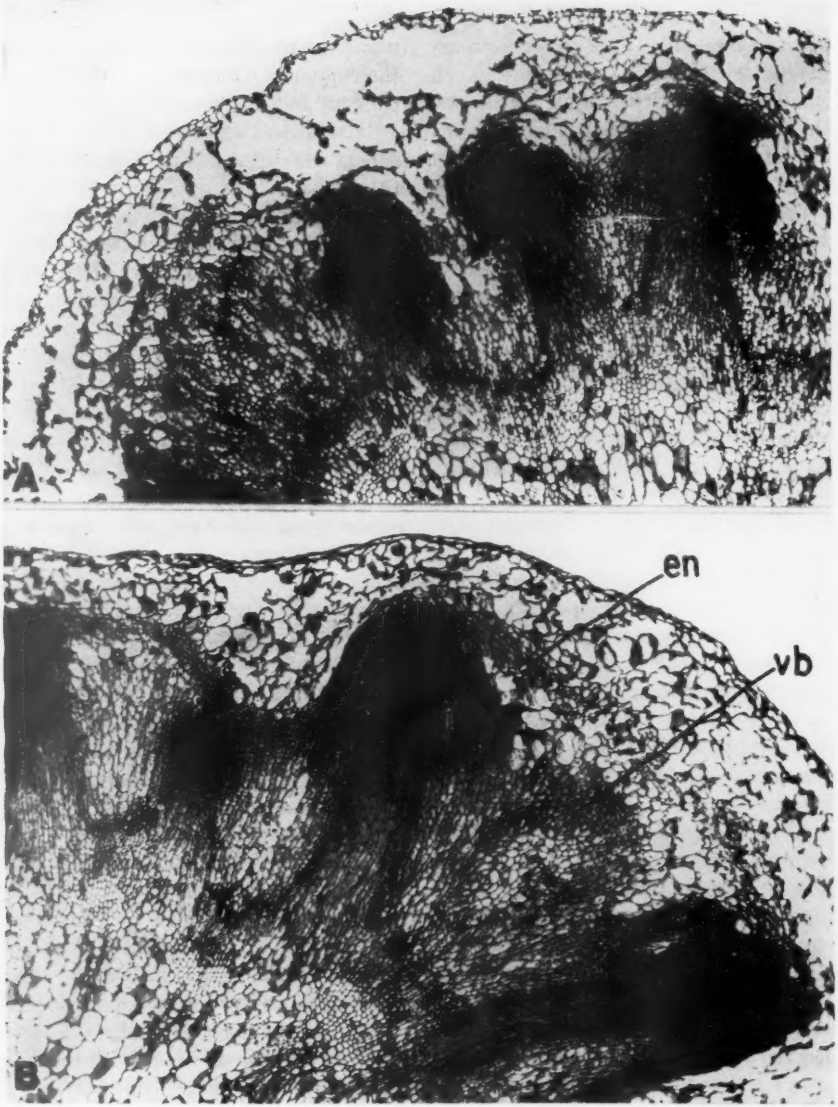

FIG. 10.—Transections of stem tips through root zone, 5 days after treatment. *A*, ammonium salt of 2,4-D, 6 mm. below cut surface. *B*, magnesium salt of 2,4-D, 3 mm. below cut surface: *en*, endodermis. In both *A* and *B* highly meristematic tissues of root primordia in rays alternating with maturing, slightly proliferated tissues of vascular bundles. Pericyclic cells enlarged over vascular bundles or maturing as fibers. Differentiation of tracheids and vascular bundles (*vb*) among derivatives of phloem parenchyma. Cells of inner portion of rays and xylem parenchyma slightly active. Except for activity in rays, degree of proliferation markedly less than in zone of major proliferation. Organization of root primordia and differentiation of proliferated cells more advanced in stem treated with magnesium salt.

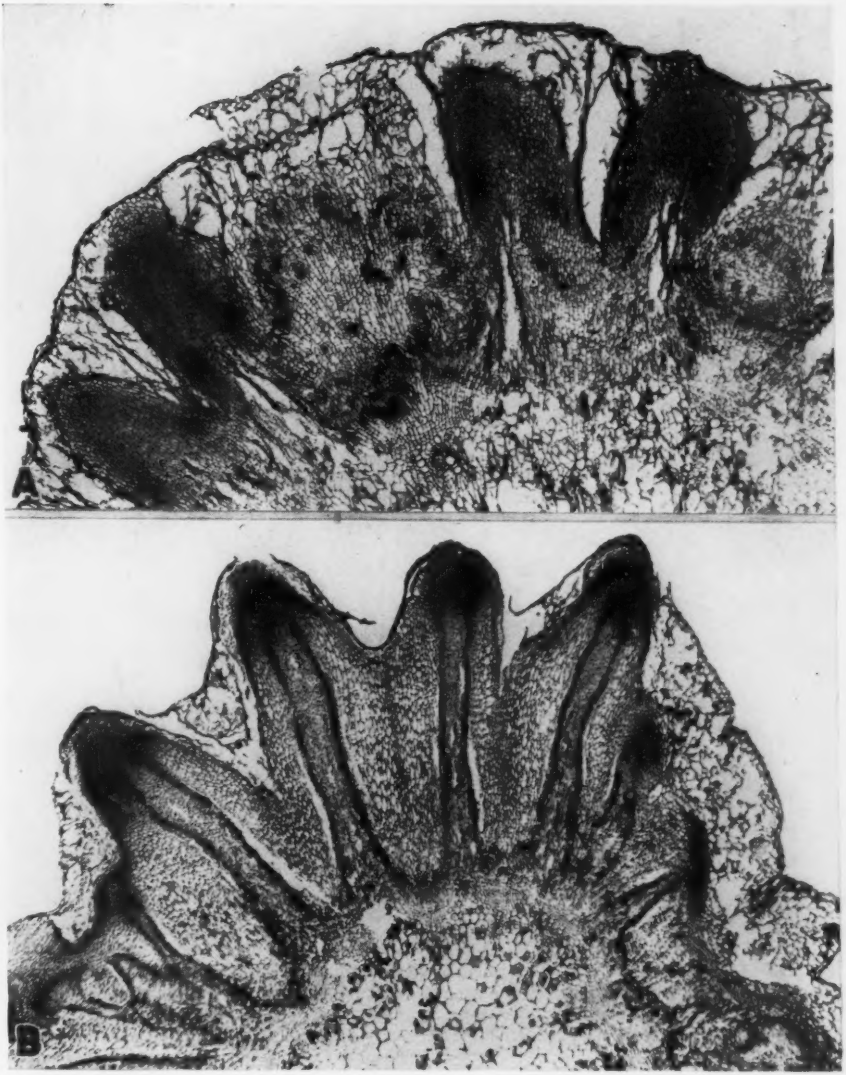

FIG. 11.—Transections of stem tips through root zone, 12 days after treatment. *A*, ammonium salt of 2,4-D, 5.5. mm. below cut surface. *B*, magnesium salt of 2,4-D, 3 mm. below cut surface. More mature stages in development of root zone. Outer tissues and many areas of proliferated inner tissues collapsed.

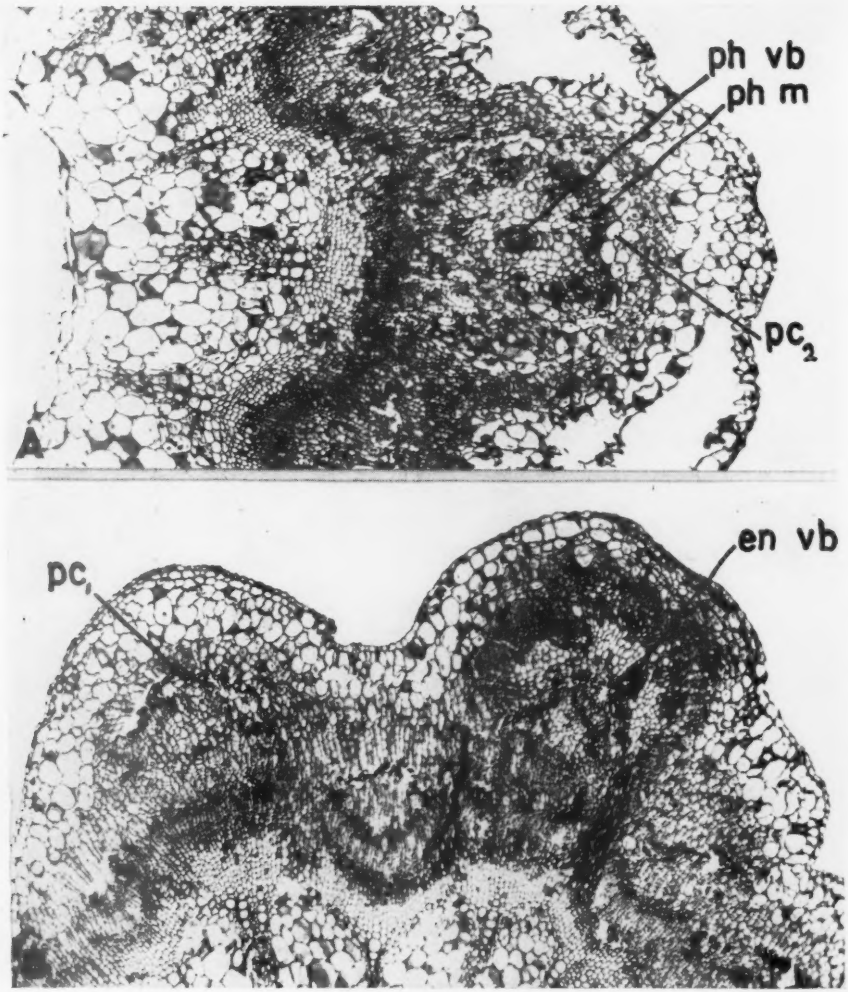

FIG. 12.—*A*, ammonium salt of 2,4-D, 7.5 mm. below cut surface; *B*, magnesium salt of 2,4-D, 5.5. mm. below cut surface. Both transections through zone of minor proliferation, 5 days after treatment. Outer cortical parenchyma generally inactive; inner cortical parenchyma and endodermis slightly proliferated and commonly maturing as parenchyma. Pericyclic cells frequently matured as fibers (pc_1), but some cells greatly enlarged and maturing as parenchyma (pc_2). Primary phloem parenchyma adjacent to pericycle remaining meristematic (*ph m*) and continuing proliferation, especially in *A;* other derivatives of proliferated phloem parenchyma maturing in complex pattern of reticulate tracheids and small vascular strands (*ph vb*). Band of cambial derivatives less wide than at upper levels, cells maturing as secondary phloem and short xylem tracheids. Slight activity in inner portion of ray, especially in *A*. Bundle to left in *B* typical of response in small bundles with both treatments. Over large bundle to right in response induced by magnesium salt outer cortical parenchyma characteristically proliferated; inner cortical parenchyma and endodermis very active with many derivatives differentiated as vascular bundles (*en vb*) or as reticulate tracheids which connect through rays to xylem.

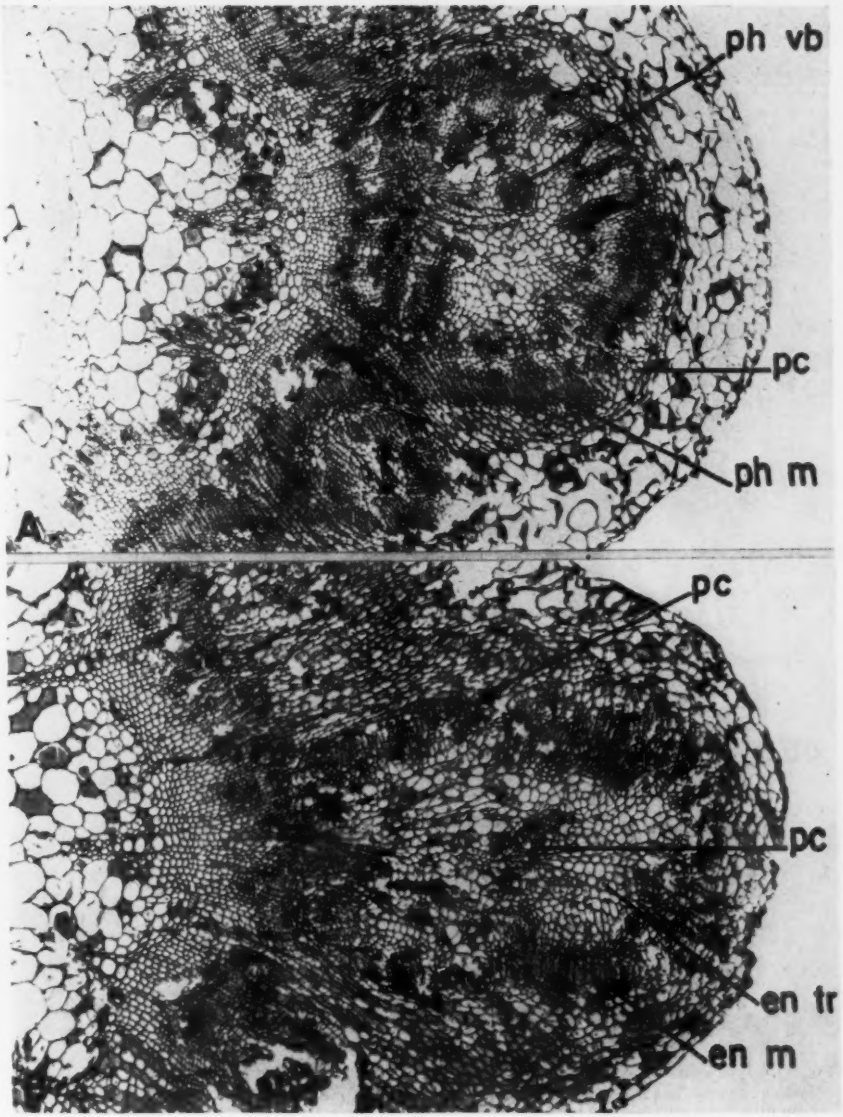

FIG. 13.—*A*, ammonium salt of 2,4-D, 8 mm. below cut surface; *B*, magnesium salt of 2,4-D, 4 mm. below cut surface. Both transections in zone of minor proliferation, 12 days after treatment: *pc*, pericycle. In general, both sections similar to those at 5 days but much more mature. In phloem and endodermis continued activity of meristematic areas (*ph m* and *en m*) and further differentiation of tracheids (*en tr*) and vascular bundles (*ph vb*) result in greater complexity of tissue patterns. Note continued proliferation of inner ray parenchyma in *A* and its absence in *B*.

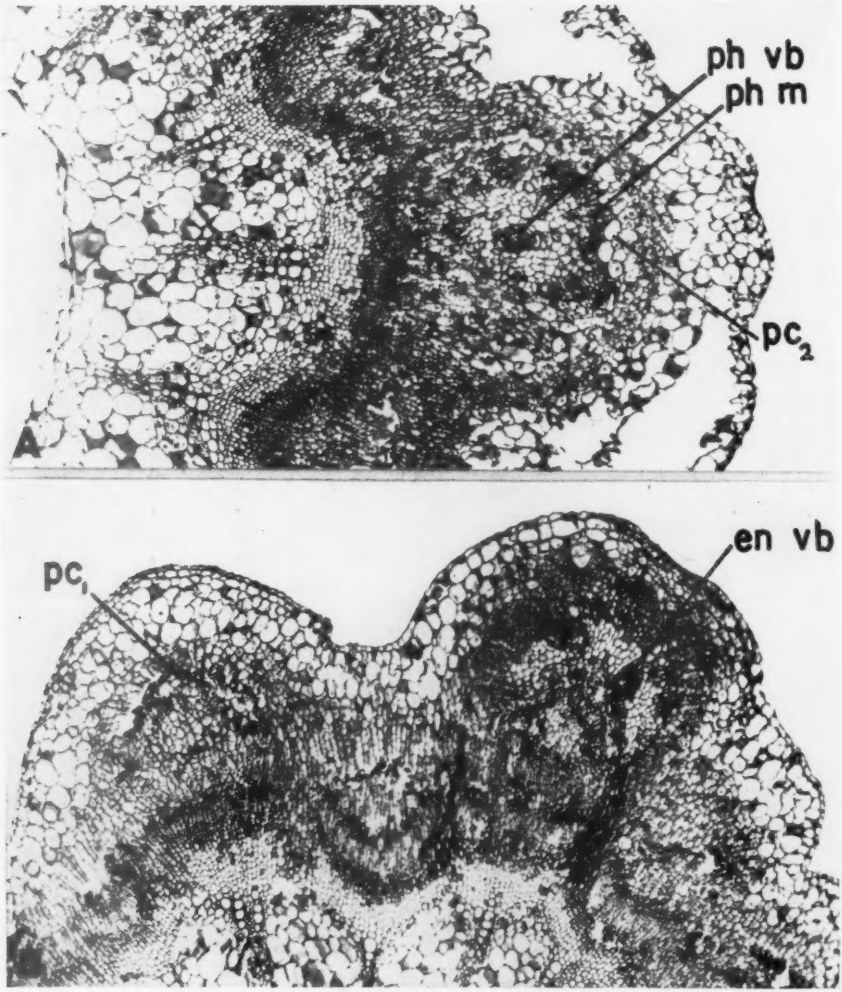

FIG. 12.—*A*, ammonium salt of 2,4-D, 7.5 mm. below cut surface; *B*, magnesium salt of 2,4-D, 5.5. mm. below cut surface. Both transections through zone of minor proliferation, 5 days after treatment. Outer cortical parenchyma generally inactive; inner cortical parenchyma and endodermis slightly proliferated and commonly maturing as parenchyma. Pericyclic cells frequently matured as fibers (pc_1), but some cells greatly enlarged and maturing as parenchyma (pc_2). Primary phloem parenchyma adjacent to pericycle remaining meristematic (*ph m*) and continuing proliferation, especially in *A;* other derivatives of proliferated phloem parenchyma maturing in complex pattern of reticulate tracheids and small vascular strands (*ph vb*). Band of cambial derivatives less wide than at upper levels, cells maturing as secondary phloem and short xylem tracheids. Slight activity in inner portion of ray, especially in *A*. Bundle to left in *B* typical of response in small bundles with both treatments. Over large bundle to right in response induced by magnesium salt outer cortical parenchyma characteristically proliferated; inner cortical parenchyma and endodermis very active with many derivatives differentiated as vascular bundles (*en vb*) or as reticulate tracheids which connect through rays to xylem.

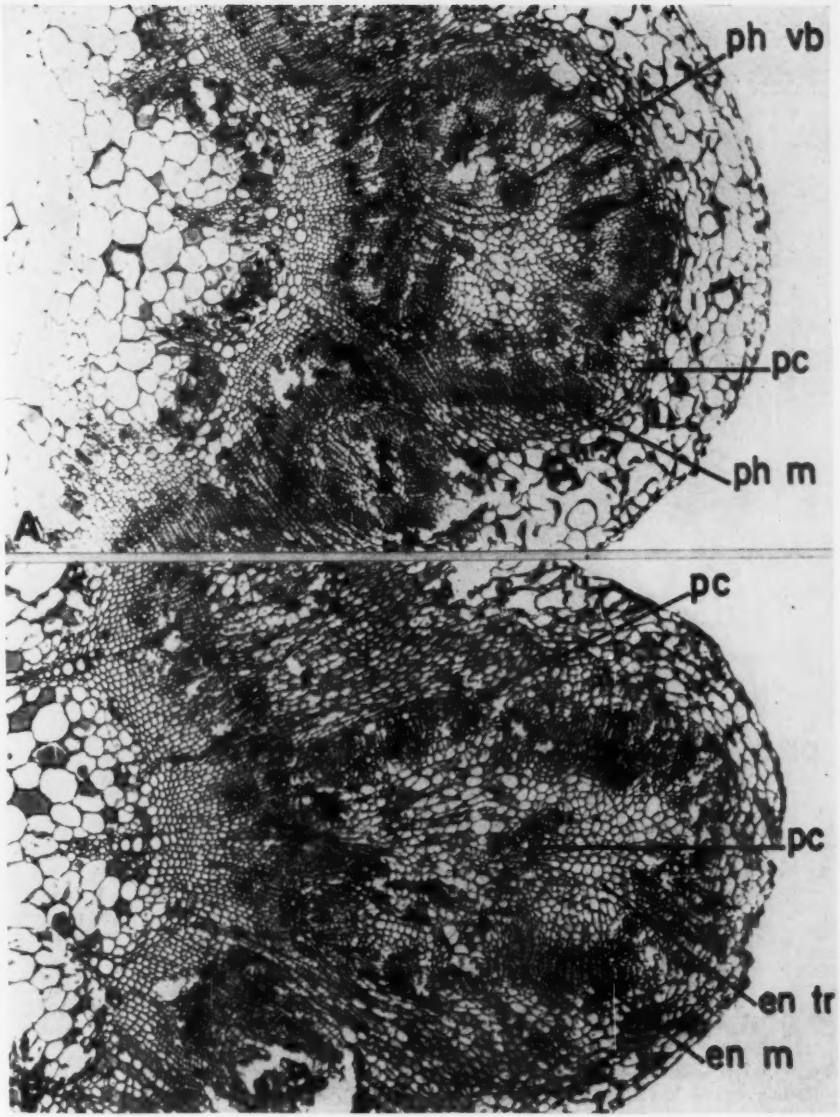

FIG. 13.—*A*, ammonium salt of 2,4-D, 8 mm. below cut surface; *B*, magnesium salt of 2,4-D, 4 mm. below cut surface. Both transections in zone of minor proliferation, 12 days after treatment: *pc*, pericycle. In general, both sections similar to those at 5 days but much more mature. In phloem and endodermis continued activity of meristematic areas (*ph m* and *en m*) and further differentiation of tracheids (*en tr*) and vascular bundles (*ph vb*) result in greater complexity of tissue patterns. Note continued proliferation of inner ray parenchyma in *A* and its absence in *B*.

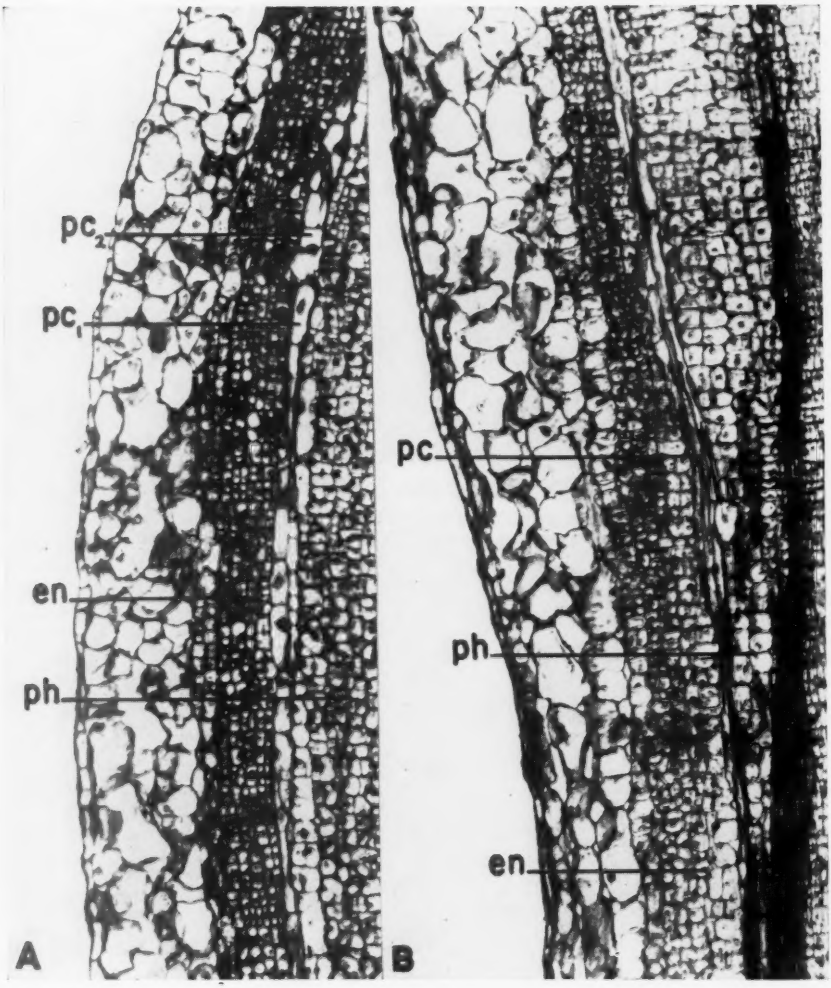

FIG. 14.—Longisections of stem tips, 3 days after treatment with copper salt of 2,4-D. *A*, detail of early proliferation at upper levels (1–3 mm.) to show response of pericyclic cells: many cells enlarged, some dividing (pc_1), others recently divided and multinucleolate (pc_2). Note character of proliferation in endodermis (*en*) and phloem parenchyma (*ph*). *B*, similar detail at lower levels (5–7 mm.). Pericyclic cells (*pc*) less active, response in phloem parenchyma (*ph*) terminating, but proliferation in endodermis (*en*) extending downward for several millimeters.

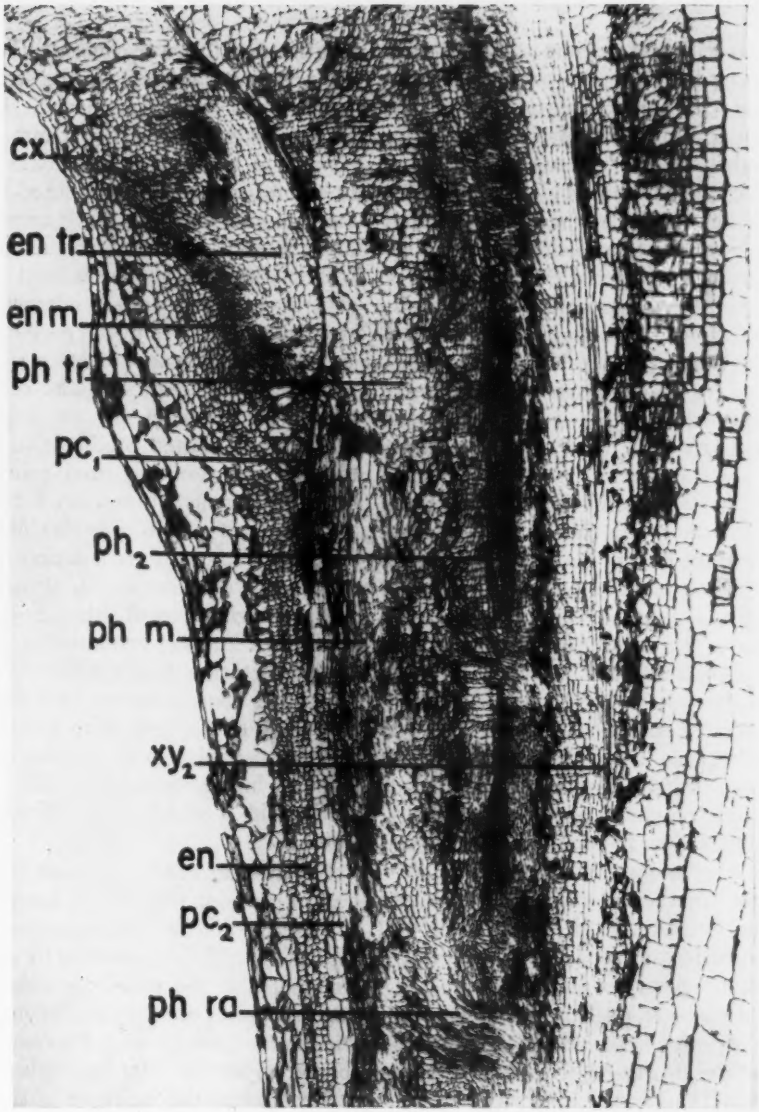

FIG. 15.—Longisection, 12 days after treatment with calcium salt of 2,4-D. Section of older stem in zone of minor proliferation (4.5–8 mm.) to illustrate in longisection complex and varying character of proliferation and maturation in large bundle. Compare with fig. 13. Cortical parenchyma (*cx*) proliferated. Endodermis strongly proliferated at upper levels and differentiating tracheids (*en tr*) or forming meristematic strands (*en m*); less active at lower levels and maturing as parenchyma (*en*). Pericyclic cells maturing as fibers (pc_1) or slightly proliferated and maturing as parenchyma (pc_2). Phloem parenchyma greatly proliferated; many derivatives maturing as tracheids (*ph tr*), strands of which may connect with xylem through phloem rays (*ph ra*); other areas parenchymatous or remaining meristematic (*ph m*). Secondary vascular tissues (ph_2 and xy_2) matured.

less so with the calcium salt, and least with 2,4-D. This pith parenchyma, together with parenchymatous cells of the xylem and inner ray, proliferated above the original cut surface in tumors induced by the calcium and magnesium salts.

In the evaluation of responses induced by the various treatments zonation is an important characteristic of the tumor. A study of the tumors on the basis of zonation disclosed well-defined differences but at the same time established a consistency of response throughout all the treatments. A shallow, flared tumor with major activity just below the cut surface and some proliferation above (magnesium salt) was readily distinguished from a deep tumor with the greatest enlargement much below the cut surface and constriction at the apex (2,4-D). Although these represented extremes of response in this experiment, comparison of equivalent zones and stages of development showed close relationship between the two types. Moreover, a progressive series between these two was formed with the other salts of 2,4-D. It is significant that comparisons of the different tumors resulted in each instance in the same series of relationships among the substances, whether the differences were expressed in histological details or in the larger pattern of zonation. This is illustrated in figures 1–5 and table 2.

The series of responses strongly suggests variation in the effective concentration of the growth-regulating substances. In decapitated bean plants with a known gradation in the concentration of indoleacetic acid (7), similar changes in the character and levels of response were observed. It was shown that responses induced at successively lower levels in a stem tip treated with a high concentration of indoleacetic acid are similar to responses at higher levels induced by treatment with lower concentrations. In the present experiment, in the stem tips treated with the magnesium salt, the responses at upper levels (0–1 mm.) were similar to those at intermediate levels (5–6 mm.) in the tumor induced by the ammonium salt (figs. 4, 5). If zonation is interpreted as an expression of concentration, the five substances used in this experiment are not equally effective. The strongest stimulation of response resulted from treatments with 2,4-D and with the ammonium salt, and the least with the magnesium salt. On this basis the zone of limited proliferation is interpreted as resulting from concentrations high enough to approach toxicity and to inhibit growth. The zone of major proliferation represents a concentration of greatest effectiveness in stimulating and prolonging growth. Roots appear to be induced at lower concentrations. Minor proliferation occurred at still lower levels and concentrations. A somewhat similar series of effects from stimulation to inhibition of growth correlated with low and high concentrations of indoleacetic acid is reported by HSUEH and LOU (4).

Explanation of the apparent concentration gradient with the different salts of 2,4-D is difficult. All substances were applied in 0.5% concentration by weight. Differences in the molecular weights of the compounds result in only slight variation in the amount of 2,4-D present and do not appear to offer an explanation, particularly as the sequence in the molecular weights does not correlate with the sequence established for effectiveness of response. Solubility was also taken into consideration but did not seem to be a direct factor. The method by which the cation changed the effective concentration and thus modified the activity of the growth-regulating substance is difficult

to analyze. Reduction in the effectiveness of the various substances may take place in numerous ways, such as the initial entrance of the substance into the plant tissues, translocation, changes in the permeability of the membranes, and the reactions within the cells.

The role of the cation in directly influencing the character of histological response is also difficult to determine. Certain histological differences may be attributable to independent action of the cation or these differences may be correlated with zonation and concentration and thus indirectly with the cation. However, much of the response appears to be a 2,4-D effect. The result of greatest significance in the present experiment is the considerable reduction in the effective concentration of the growth-regulating substance according to the kind of salt of 2,4-D in which it is applied.

Summary

1. Young kidney-bean plants were decapitated in the second internode. 2,4-Dichlorophenoxyacetic acid or one of four salts—ammonium, copper, calcium, or magnesium 2,4-dichlorophenoxyacetate—was applied to the cut surface in

TABLE 2

ZONATION IN TUMORS INDUCED BY 2,4-DICHLOROPHENOXYACETIC ACID AND FOUR OF ITS SALTS, 12 DAYS AFTER TREATMENT

Depth in stem (mm.)	2,4-D	Ammonium salt	Copper salt	Calcium salt	Magnesium salt
Cut surface					
0	Limited proliferation	Limited proliferation	Limited proliferation	Major proliferation	Major proliferation
1					
2			Major proliferation		Root formation
3		Major proliferation		Root formation	
4	Major proliferation		Root formation		Minor proliferation
5		Root formation		Minor proliferation	
6	Root formation		Minor proliferation		
7		Minor proliferation			
8	Minor proliferation				
9					
10					
11					
12					
13					
14					
15					

0.5% concentration by weight in lanolin paste. Observations were made of material grown for a period of 30 days after treatment.

2. Gross response showed characteristic differences among the tumors. Treatments with 2,4-D and with the ammonium salt resulted in relatively deep, club-shaped tumors. Proliferation was entirely below the cut surface. The tumor induced by the copper salt was likewise formed below the cut surface but was somewhat shallower. Those induced by calcium and magnesium salts were broadly flared at the cut surface and gradually tapered below. Growth above the cut surface occurred with both of the latter substances but to a more marked degree with the magnesium salt.

3. Histological responses in the early stages showed considerable similarity. In parenchymatous tissues of the inner cortex including the endodermis, of the pericycle, phloem, cambial zone, xylem, and rays, proliferation was initiated soon after treatment. Response induced by the magnesium salt was earlier and more marked than in other treatments, less so with the calcium salt, and least with the ammonium salt and with 2,4-D.

4. Study of the tumor as a whole disclosed a pattern of zonation for all treatments. At upper levels a zone of limited proliferation was found in the responses to 2,4-D and to the ammonium and copper salts. This zone was lacking in the other responses. A zone of major proliferation occupied the intermediate levels below the zone of limited proliferation in treatments with 2,4-D and with the ammonium and copper salts but was found at upper levels in the tumors induced by the calcium and magnesium salts. In response to the magnesium salt this zone was only poorly represented. With all substances a root zone was formed at next lower levels, and below the roots a zone of minor proliferation terminated the tumor. Comparison on the basis of zonation disclosed a consistent sequence among the substances according to the characteristics of the responses induced: 2,4-D and the ammonium, copper, calcium, and magnesium salts. This same series was duplicated in gross responses and in details of histological responses.

5. Maturation was earliest and most marked in the treatment with the magnesium salt and least with the ammonium salt and with 2,4-D. In the zone of limited proliferation maturation occurred early, was mainly as parenchyma, and was soon followed by collapse. Maturation in the zone of major proliferation was longest delayed. Many of the derivatives differentiated as parenchyma except at levels of transition to lower zones. In the root zone the rays organized as root primordia remained active until the death of the tumor 3 weeks or more after treatment. Proliferated tissues of vascular bundles and associated endodermis and cortical parenchyma both in the root zone and in the zone of minor proliferation exhibited complicated patterns of differentiation into tracheids, parenchyma, vascular bundles, and meristematic areas.

6. Characteristic differences in histological responses to the different substances are discussed. The most pronounced differences were found in decapitated stems treated with the magnesium salt. However, no response induced by one substance was entirely absent in the reaction to another substance. All responses appeared to fall within the range of effects distinctive for 2,4-D. Independent effects of the different cations on stem responses were also considered.

7. Zonation is interpreted as an expression of a concentration gradient. On the basis of this interpretation the effectiveness of the growth-regulating substance was greatly modified by the form of the salt which was applied.

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A RAPID SENSITIVE METHOD FOR DETERMINATION OF LOW CONCENTRATIONS OF 2,4-DICHLOROPHENOXYACETIC ACID IN AQUEOUS SOLUTION[1]

DANIEL READY[2] AND VIRGINIA Q. GRANT[3]

Introduction

In physiological and herbicidal studies on 2,4-dichlorophenoxyacetic acid (2,4-D) there is frequently a need for determining the biological activity of small amounts of this compound in aqueous solution. No satisfactory chemical method has yet been devised, primarily because this compound is relatively inert chemically.

Recently BANDURSKI (1) described a spectrophotometric method for determining 2,4-D in aqueous and ether solutions in which Beer's law was found to hold within 5% limits over a concentration range of 6–250γ/ml. This method is based on the absorption of light in the ultraviolet region. Earlier SWANSON (3) devised a bio-assay method based on the inhibition of further growth of the primary root of surface-sterilized and pregerminated corn seeds. Within the limits of 0.1–2.0 p.p.m., reproducible and reasonably accurate results were obtained.

[1] Studies conducted at Camp Detrick, Frederick, Maryland, from April to June, 1946.

[2] Biochemist; [3] botanist.

The sensitivity of other germinating seeds to 2,4-D has been examined in an attempt to extend the range of application of the bio-assay procedure based on this principle. Representative species of the families Leguminosae, Compositae, Gramineae, Cucurbitaceae, Liliaceae, Malvaceae, and Polygonaceae were tested, and it was found that a member of the Cucurbitaceae, the common cucumber (*Cucumis sativus*), is very sensitive to 2,4-D. A detailed study was therefore made of the relationship between the degree of primary root and shoot inhibition of germinating cucumber seed and small concentrations of 2,4-D; the results form the basis of this paper.

Material and methods

The cucumber seed used was a commercially available variety, Early Fortune. Six-inch Petri dishes were fitted with circles of filter paper and to each were added 15 ml. of the solution of 2,4-D under test. Twenty-five cucumber seeds were placed in each dish, and five replicate dishes were employed for each of fifteen different concentrations. Untreated controls were set up using 15 ml. of distilled water. The dishes were then covered and placed in a dark constant-temperature room at 28° C. At the end of 96 hours they were removed, and lengths of the primary root and shoot were measured.

Results

The concentrations employed ranged from 0.001 to 10.0 p.p.m. Examination of the data (tables 1, 2, figs. 1–3) shows that there was a simple quantitative relationship between the concentrations of 2,4-D used and the degree of inhibition of growth of primary roots and shoots of the germinating cucumber seed. Calculation of the F values for these data showed high statistical significance for treatment and no significant difference for replication.

The roots were extremely sensitive to minute amounts of this acid, concentrations as low as 0.005 p.p.m. having produced inhibition to a degree which was statistically significant when compared with the control (table 1). All concentrations above this point to the maximum of 10 p.p.m. produced growth inhibition highly significant at the 1% level when compared with the control. Differences between treatments were also highly significant at concentrations from 0.01 to 0.10 p.p.m. At a concentration of 1.0 p.p.m. inhibition of growth had essentially reached its maximum, and the curve leveled off. Consequently, inhibition of growth of the primary root offers an excellent means of determining 2,4-D in concentrations of 0.005–1.0 p.p.m.

The shoots treated with 0.01 p.p.m. (table 2) showed appreciable growth inhibition when compared with the control, but the differences between treatments were not statistically significant until a concentration of 0.50 p.p.m. was reached. Differences between treatments were highly significant between 0.5 and 5.0 p.p.m. with the exception of the 1.0-p.p.m. concentration. Above 5.0 p.p.m. maximum inhibition of shoot development had been practically attained, and the curve leveled off. Consequently, measurement of the shoot offers an additional means for determining concentrations of 2,4-D from 0.5 to 5.0 p.p.m.

When the values for root and shoot inhibition were plotted against log concentrations of 2,4-D, straight lines were obtained for which equations fitting closely were derived (fig. 3). The slopes of the lines are not quite identical.

TABLE 1

INHIBITION OF PRIMARY ROOTS OF GERMINATING CUCUMBER SEEDS EXPOSED FOR 96 HOURS TO DIFFERENT CONCENTRATIONS OF 2,4-D

Concentration of acid (p.p.m.)	Mean lengths in replicate (mm.)					Mean*	% of control
	1	2	3	4	5		
0 (control)	70	62	58	67	57	62.8	.....
0.001	60	66	60	55	59	60.0	95.5
0.005	57	61	56	62	55	58.2	92.6
0.010	44	37	49	45	42	43.4	69.1
0.025	38	35	36	36	42	37.4	59.5
0.050	29	33	27	28	34	30.2	48.0
0.075	23	26	26	27	24	25.2	40.1
0.10	14	18	20	18	29	19.8	31.5
0.25	18	15	17	18	16	16.8	26.7
0.50	12	13	12	13	14	12.8	20.3
0.75	9	10	12	11	10	10.4	16.5
1.0	8	8	13	9	10	9.6	15.2
2.5	7	8	6	7	9	7.4	11.7
5.0	6	7	6	7	7	6.6	10.5
7.5	6	7	6	7	6	6.4	10.1
10.0	6	6	6	6	6	6.0	9.5

*Minimum significant difference between means of concentrations at 5% level of probability is 3.82 mm.; at 1% level, 5.08 mm.

TABLE 2

INHIBITION OF SHOOTS OF GERMINATING CUCUMBER SEEDS EXPOSED FOR 96 HOURS TO DIFFERENT CONCENTRATIONS OF 2,4-D

Concentration of acid (p.p.m.)	Mean lengths in replicate (mm.)					Mean*	% of control
	1	2	3	4	5		
0 (control)	60	56	53	60	59	57.6	.....
0.001	53	53	56	55	57	54.8	95.1
0.005	52	54	50	56	54	53.2	92.3
0.010	50	52	49	50	47	49.6	86.1
0.025	49	51	46	53	55	50.8	88.1
0.050	45	52	45	50	54	49.2	85.4
0.075	52	48	47	48	55	50.0	86.8
0.10	53	47	56	51	48	51.0	88.5
0.25	50	45	48	41	50	46.8	81.2
0.50	44	43	39	47	44	43.4	75.3
0.75	38	37	37	38	38	37.6	65.2
1.0	37	33	37	40	37	36.8	63.8
2.5	31	28	27	26	28	28.0	48.6
5.0	18	23	20	22	19	20.4	35.4
7.5	14	15	15	18	17	15.8	27.4
10.0	13	14	16	14	13	14.0	24.3

*Minimum significant difference between the means of concentration at 5% level of probability is 3.34 mm.; at 1% level, 4.44 mm.

Discussion

The need for a sensitive and accurate method for the determination of small amounts of 2,4-D in aqueous solution has been adequately met through the activity of this acid in inhibiting the primary root and shoot of germinating cucumber seed. Such activity is so quantitatively related to the concentrations of acid employed that this fact serves as a means of determining the amounts of acid present. Differing sensitivities of the root and shoot to the same concentration of the acid present a desirable condition whereby, in the case of an unknown solution, either the root or the shoot, or both in the case of the 0.50–1.0 p.p.m. range, may be used to determine the acid concentration.

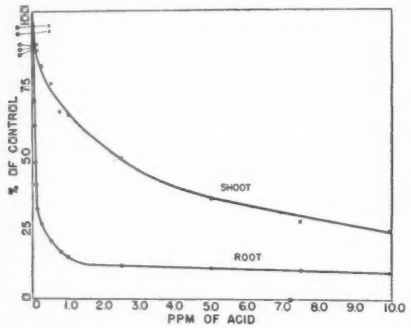

FIG. 1.—Inhibition of growth of primary roots and shoots of germinating cucumber seed exposed for 96 hours to different concentrations of 2,4-D.

Furthermore, this method is simple and rapid. No surface sterilization of the seed was found to be required, since in this study and in hundreds of other determinations fungal contamination was very infrequent, even though untreated seed only was used. In addition, no initial germination and subsequent selection of the seed are necessary before treatment as in the method described by SWANSON (3). It can be performed with a minimum of equipment and could undoubtedly be employed in the field, provided a reasonably constant-temperature storage chamber is available near by. By measuring the activity on germinating cucumber seeds, concentrations from 0.005 to 5.0 p.p.m. can be determined accurately by this method, and dilution to this range will permit determination of more concentrated solutions. Solutions of 2,4-D of unknown concentration, after dilution, may be subjected to the procedure herein described, and growth inhibition may be expressed in percentage of control. This may then be compared with the standard curve, and the presumptive concentration of 2,4-D may be thereby ascertained.

The linear character of the relationship between inhibition of both roots and shoots of the cucumber seedling and concentrations of 2,4-D considered on a logarithmic basis may throw some light on the mechanism of action of a growth-regulating substance of this type. It is not known whether the over-all inhibition produced is the result of a simple effect on some cellular mechanism, or

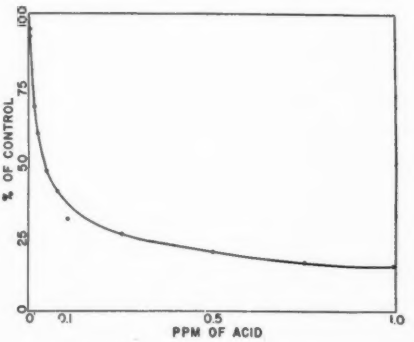

FIG. 2.—Inhibition of growth of primary roots of germinating cucumber seed exposed for 96 hours to different concentrations of 2,4-D.

whether it is a summation of effects on various growth mechanisms. The latter is the more probable, since the magnitudes of the responses produced in root and shoot are not identical. However, as far as each organ is concerned, the effect is proportional to concentration, and the relationship can apparently be treated as a single reaction.

The procedure thus developed is predicated entirely upon the activity of 2,4-D on germinating cucumber seeds in aqueous media. Recently LUCAS and HAMNER (2), using onion extract, found that activation of 2,4-D was obtained through a synergistic effect; they also found that dilution of the extract increased this synergistic effect. Some lesser synergistic effect was evidenced in the case of garlic extract, whereas none was found with tomato extract. In work under progress at this installation, inhibition of germinating cucumber seed by some factor present in corn-plant extract has been noted. On the other hand, soil leachates have been examined for 2,4-D content by this method, and, after suitable standard curves had been established by adding known amounts of 2,4-D to the leachate from untreated soil, good agreement was obtained between the theoretical amounts of 2,4-D added to the soils and recovery. Also, determinations on artesian waters to which known amounts of 2,4-D had been added were in good agreement with the standard curve. From the foregoing it is obvious that in certain types of biological materials the terms "activity" (or inhibition) and "concentration" may not be used synonymously, and, consequently, application of the procedure described in this paper would be relative rather than absolute. The basic purpose of this paper, however, is to describe a quantitative method for the determination of 2,4-D in aqueous solutions which can then be modified as required for the determination of this acid in other media, such as by constructing standard curves using such media as a base, or by the inclusion of appropriate blanks.

Further investigations are under way to determine the suitability of this method for the quantitative estimation of activity of small amounts of other members of the phenoxyacetic acid series (4). Included in this group are 4-chlorophenoxyacetic acid, 2-methyl-4-chlorophenoxyacetic acid, and 2,4,5-trichlorophenoxyacetic acid. Preliminary results appear encouraging and publication of results of detailed tests on these compounds will be made shortly.

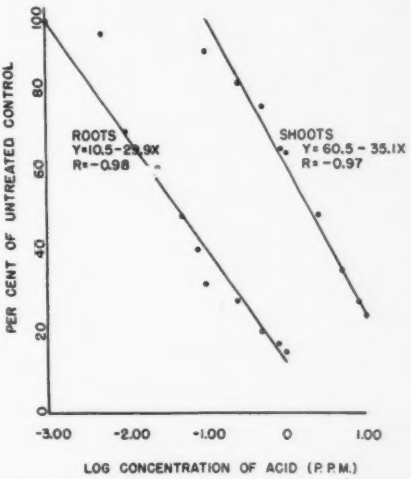

FIG. 3.—Regression equations and coefficients of correlation of roots and shoot lengths of germinating cucumber seed exposed to 2,4-D.

Summary

1. A simple and rapid quantitative method for the determination of small amounts of 2,4-dichlorophenoxyacetic acid (2,4-D) in aqueous solution is de-

scribed. This method is based on the activity of the acid in inhibiting the growth of the primary root and shoot of germinating cucumber seed. It is sensitive to 0.005 p.p.m. and is applicable from this concentration to 5.0 p.p.m. Initial surface sterilization of the seed, selection of pregerminated seed of certain root lengths, and special equipment are not necessary.

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VEGETATIVE RESPONSES OF BROMUS INERMIS TO CERTAIN VARIATIONS IN ENVIRONMENT[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 587

JOHN C. DIBBERN

Introduction

Smooth brome grass (*Bromus inermis* Leyss.) has long been cultivated in Hungary and Russia. The first recorded importation of seed into the United States was in 1880, and many importations from different sources have been made since. From these importations our present strains have been developed.

The species is now found as far south as Tennessee, Kansas, and California and extends northward through Canada into Alaska. On mountain ranges in the semiarid regions of the West and Northwest it is frequently found at all elevations up to 9,000 feet, and in central Utah up to 10,500 feet, though it does not reseed at this elevation (3). It is important as a hay and pasture grass, especially from Minnesota and Kansas to eastern Oregon and Washington, occasionally eastward to Michigan and Ohio; and in the artificial reseeding of denuded range land in regions of light rainfall and moderate summer temperatures.

Smooth brome is a perennial and spreads vegetatively by means of rhizomes. It grows rapidly in the spring and, though remaining green throughout the warm summer months, then makes very little additional growth. Inflorescences develop in the spring, and anthesis occurs in early summer. Through environmental selection northern and southern strains have evolved, these strains maturing late and early, respectively, when grown in the latitude of Lincoln, Nebraska (8). Within these strains the individual plants show a great diversity of growth habit, ranging from bunch types to semicreepers.

A study has been made of some of the vegetative responses of diverse plants of smooth brome grass to variations in clipping frequency, soil temperature,

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

shading, and container size in an attempt to shed some light on (*a*) the ability of the species to withstand grazing or mowing; (*b*) its limitation in distribution to only the higher latitudes; (*c*) its shade tolerance; and (*d*) the type of container that will allow maximum growth in the greenhouse. The experiments were conducted in the greenhouses and garden of the University of Chicago from the spring of 1946 through early summer, 1947.

Material and methods

Because of the diversity of the species, it seemed desirable to work with a wide variety of plants. To this end the clonal and seed materials used represent plants originating from Saskatchewan to Washington, Kansas.

In greenhouse experiments the soil used consisted of three parts loam soil of prairie origin mixed with one part sand. A supplementary nitrate solution was occasionally applied and consisted of 3.5 gm. $Ca(NO_3)_2 \cdot 4H_2O$ per liter of water.

In experiments where both root and top weights are reported, the separation of tops and roots was made on a morphological basis rather than at the ground level. Underground stem parts were thus considered part of the tops. Where root weights were not taken, tops were clipped at a height of 2 inches above the ground level except where specifically stated otherwise.

Stem counts represent only those stems that had emerged from the soil, including both elongated and unelongated tillers, but do not include dead stems.

Experimentation

Clipping

Greenhouse series.—On April 27 and 28, 1946, six divisions of each of twenty-three clones which had been growing outdoors either at Chicago, Illinois, or at Madison, Wisconsin, were planted separately in 8-inch unglazed clay pots on well-illuminated benches in the greenhouse. Each division consisted of approximately six stems and accompanying roots. The leaves of plants that showed severe wilting after planting were clipped to decrease water loss, but in no instance was the clipping severe enough to remove entire leaf blades or growing points. Nitrate solution (192 ml.) was supplied to each plant on May 4, 1946, September 5, 1946, and April 3, 1947, and they were watered when necessary.

On June 30, 1946, the three most uniform divisions of each clone were selected for experimentation, but only the seventeen clones in which all three divisions survived until the termination of the experiment are reported upon. Series C was an unclipped control, series 1 was clipped on June 30, 1946, and series 2 on June 30 and September 27.

Because of general lack of vigor, a final harvest was considered inadvisable in the fall, and the effects of clipping for the 1946 season are therefore shown only by stem counts (table 1).

The clipping on June 30 caused a reduction in the number of stems in series 1 to 76% as measured on July 13, but series 2 was reduced to only 86%. The stem counts and top weights on June 30 were larger in series 2 than in series 1, and it would thus appear that the larger plants were less affected by clipping than were the smaller ones.

On September 27 the stem count of series 1 was 2% below the count on June 30, and that of series 2 was 23% above. The yield of tops in series 2, however, was less than half that of June 30. The lower yield between June 30 and September 27 cannot be considered entirely an effect of the first clipping. Growing con-

ditions in the greenhouse during this period were quite adverse. Daytime soil temperatures averaged 23° C., and maximum air temperatures 33° C. The plants also became infested with mealy bugs, aphids, and red spiders, and, in addition to the injuries so caused, there was some injury from control spraying.

On November 24, stem counts showed the control to have increased 97% over the value on June 30, series 1 had increased 12%, and series 2 had decreased 8%. During the period from November 24, 1946, to January 18, 1947, the light intensity was quite low in the greenhouse, and all three series showed a decrease in the number of live stems.

By March 31, 1947, the plants had resumed active growth, and both clipped series showed an increase in stem numbers over the January count. However, the stem count of series C showed a decrease from the January count. This decrease might indicate that the volume of soil in the 8-inch clay pots had become a limiting factor to further increase in size, as suggested by the results with different-sized containers (see soil-volume experiment). On April 25 the three series were clipped back to 2 inches. The relative yields produced between April 25 and June 2, and the relative numbers of stems on June 2, 1947, when the experiment was terminated, are taken as a measure of the aftereffects of no clipping, one clipping, and two clippings the previous year. If the top growth produced between April 25 and June 2 is converted to dry-weight yield per stem based on the counts of June 30, 1946, the value for series 1 is 23% less, and that of series 2 is 43% less, than the control. The stem counts on June 2, 1947, show that series C had 80% more and series 2 had 8% less than on June 30, 1946.

HARRISON and CRAWFORD (5) found in a field experiment with smooth brome that applications of ammonium sulfate had more effect upon stem size than upon total number of stems in determining yield of forage. The total dry-weight yields in this clipping experiment, how-

TABLE 1

STEM COUNTS AND DRY WEIGHTS OF TOPS IN GREENHOUSE CLIPPING EXPERIMENT

Series	1946				1947			1946		1947	
	6/30	7/13	9/27	11/24	1/18	3/31	6/2	6/30	9/27	4/25	6/2
	No. of stems*							Dry wt. of tops (gm.)*			
C	292	378	530	575	559	540	527	.....	.....	46.19	35.20
1	246	186	241	276	270	316	290	59.14	.....	30.59	22.87
2	305	263	375	280	269	278	282	74.46	35.28	19.94	20.71
	No. of stems in % of count on 6/30							Dry wt. per stem (gm.)			
C	100	129	182	197	192	185	180	.....	.....	0.086	0.067
1	100	76	98	112	110	128	118	0.240	.....	.097	.079
2	100	86	123	92	88	91	92	0.244	0.094	0.072	0.073

* Sums of values for seventeen clones.

ever, showed much closer correlation with stem counts than with average weight per stem (table 1).

GARDEN SERIES.—On April 25, 1946, six divisions of each of twenty-two clones were planted in six plots laid out side by side in an east-west direction in the garden. Each division consisted of approximately six stems and their roots and was taken from plants growing outdoors either at Chicago, Illinois, or at Madison, Wisconsin. The leaves of plants that showed severe wilting after planting were clipped to decrease water loss, but in no instance was this clipping severe enough to remove entire leaf blades or growing-points. The divisions were spaced 18 inches apart in the east-west direction and 24 inches apart in the north-south. The plants were watered sufficiently to prevent deficiency at any time. On June 24, 1946, the three most uniform divisions of each clone were selected for the clipping experiment.

Series C was an unclipped control, series 1 was clipped on June 24, and series 2 on June 24 and August 23. On November 15 all three series were clipped back to 2 inches and the total oven-dry yield of tops was determined. On June 7, 1947, the three series were clipped again to 2 inches, and the relative dry-weight yields were used as a measure of the aftereffects of clipping treatments in the preceding year.

The first stem count was made on June 24, 1946, at the time series 1 and series 2 were clipped the first time. The larger dry weight of tops which had been produced by series 2 was accounted for partially by the greater number of stems and partially by the greater weight per stem (table 2).

On August 23, when series 2 was clipped a second time, the numbers of stems in series 1 and series 2 were substantially greater than the numbers on June 24, though less than in the control. In contrast to the low yield from the second clipping of plants in the greenhouse, the second clipping in the garden yielded several times as much dry matter as the first, and the weight per stem was over 50% greater (table 2). This contrast is quite sharp and, even though there were five additional clones included in the garden experiment, emphasizes the differences between growing conditions outdoors and in the greenhouse.

On November 15 a stem count was made, and all three series were clipped to determine the total yields in 1946. The relative stem count was 27% less in series 1 and 55% less in series 2 than the control. The weight per harvested stem was 5% less in series 1 and 43% less in series 2 than the control. The total top weight per original stem (based on count of June 24) was 24% less in series 1 and 30% less in series 2 than the control.

From the clipping of June 7, 1947, the dry-weight yield per original stem (count of June 24, 1946) was 21% less in series 1 and 56% less in series 2 than the control.

Although these plants which grew outdoors made a much more vigorous growth than those in the greenhouse (tables 1 and 2), as measured by stem counts, top yields, and individual stem weights, the aftereffects of clipping the preceding season are very similar in greenhouse and garden as measured by the percentage differences between the 1947 yields per stem (count of June 24 and June 30, 1946) in series C, series 1, and series 2.

SEEDLINGS.—On April 30, 1947, five 8-inch glazed pots were planted with caryopses of a southern strain (no. 25); five with a strain of intermediate latitude (no. 27); and five with a northern strain (no. 26). The caryopses were covered

with $\frac{1}{4}$ inch of sifted soil, and the pots were placed on a well-illuminated bench in the greenhouse. The seedlings were watered whenever necessary to prevent water deficit and were thinned to ten per pot on May 27. On June 2, one pot (no. 1) of each strain was harvested, and oven-dry top and root weights were determined. On the same day the plants in pots 2, 3, and 4 of each strain were clipped back to 4 inches high. On June 8, pot no. 2 was harvested and pot no. 3 was again clipped back to 4 inches. On June 14 the remaining pots (nos. 3, 4, and 5) were harvested, and oven-dry top and root weights were obtained (table 3).

In all three strains the top-weight/root-weight ratio (T/R) of unclipped seedlings harvested on June 2 was greater than for unclipped seedlings harvested 12 days later (pots 1 and 5). The increase in root weight in proportion to top weight as the seedling matures results from the increasing synthesis of carbohydrate by the photosynthetic tissue, which appears to have first priority on the seed reserves. This point is emphasized, since it is pertinent to the effects of clipping upon top and root development.

Pot no. 2 of each strain, harvested 6 days following clipping on June 2, showed a higher T/R ratio in the southern and northern strains, but a lower T/R ratio in the central strain, than pot no. 1, which was harvested without being clipped (table 3).

TABLE 2

STEM COUNTS AND DRY WEIGHTS OF TOPS IN GARDEN CLIPPING EXPERIMENT

Series	1946				1947	1946				1947
	6/24	7/13	8/23	11/15	5/12	6/24	8/23	11/15	Total	6/7
	No. of stems*					Dry wt. of tops (gm.)*				
C	357	665	1896	8401	8127	.....	.....	1768.66	1768.66	3607.5
1	302	344	1169	5213	5252	72.43	.....	1062.03	1134.46	2412.5
2	434	505	1769	4675	4784	129.17	828.79	550.36	1508.32	1965.0
	No. of stems in % of count on 6/24					Dry wt. per stem (gm.)				
C	100	186	531	2375	2276	.....	.....	0.21	.....	.....
1	100	114	386	1726	1739	0.24	.....	.20	.....	.....
2	100	116	408	1077	1102	0.30	0.47	0.12	.....	.....

* Sums of values for twenty-two clones.

Plants clipped twice (pot 3) and once (pot 4) before harvesting produced less total top weight than the control (pot 5). The effect of clipping the tops in limiting the growth of roots was even greater than it was upon the tops and is reflected in the T/R ratios. In strains 25 and 27 the root weight in pot 3 harvested on June 14 is approximately the same as in pot 1 harvested on June 2—no net increase in root weight in 12 days when the tops were clipped twice.

DISCUSSION.—The results of both the greenhouse and the garden clipping experiments show conclusively that both the top yield per original stem and the num-

ber of stems were inversely proportional to the frequency of clipping and that these effects were carried over into the following growing season. With *Bromus inermis* HARRISON and HODGSON (6) also found that frequent and close clipping reduced both the amount of root growth and the total yield of tops and that, when the plants were completely defoliated, new top growth was developed in a large measure at the expense of previously deposited root reserves. MCCARTY and PRICE (7) found that the quantity of reserve carbohydrate stored in roots and stem bases of mountain brome and slender wheatgrass growing on the Wasatch Plateau of Utah was related to the amount of foliage present during the normal storage period of August and September and was less when the interval between earlier clipping and the normal storage period was shortened. Their results also indicated that the amount of carbohydrate reserves was influenced less by the frequency than by time and degree of clipping. The period of reproduction from flower-stalk elongation to formation of ripe seed is critical, and clipping at this time seems to limit subsequent storage.

The direct cause of the limitation of top growth resulting from clipping is related primarily to the reduction in photosynthetic tissue and to the use of reserves in the growth of new tops but is probably also markedly influenced by the concomitant limitation on root growth. The seedling experiment in the greenhouse showed that root growth was even more severely affected by clipping than was top growth. ROBERTSON (9) also found with *B. inermis* that, as a result of repeated clipping of tops, the rate of root growth diminished gradually until it ceased entirely, followed by a dying back from the root tips.

TABLE 3

DRY WEIGHTS OF TOPS AND ROOTS AND T/R RATIOS FOR SEEDLING CLIPPING EXPERIMENT*

STRAIN	POT NO.	TOP WT. (GM.)				ROOT WT. (GM.)	T/R
		6/2	6/8	6/14	Total	6/14	
25	1	0.55	.....	.....	0.55†	0.12†	4.6†
	2	0.35‡	0.47	.....	0.82§	0.14§	5.9§
	3	0.30‡	0.13‡	0.37	0.80	0.12	6.7
	4	0.30‡	.....	0.83	1.13	0.22	5.1
	5	.....	.....	1.89	1.89	0.61	3.1
27	1	0.64	.....	.....	0.64†	0.12†	5.3†
	2	0.36‡	0.53	.....	0.89§	0.18§	4.9§
	3	0.35‡	0.18‡	0.41	0.94	0.13	7.2
	4	0.38‡	.....	0.84	1.22	0.26	4.7
	5	.....	.....	2.17	2.17	0.62	3.5
26	1	0.30	.....	.....	0.30†	0.07†	4.3†
	2	0.22‡	0.48	.....	0.70§	0.13§	5.4§
	3	0.20‡	0.16‡	0.49	0.85	0.20	4.2
	4	0.21‡	.....	0.67	0.88	0.22	4.0
	5	.....	.....	1.46	1.46	0.52	2.8

* Values are sums of ten plants in each pot.
† On June 2.
‡ Clipped to 4 inches.
§ On June 8.

The findings of numerous workers (1, 2, 12) indicate that the greater yield of unclipped grass over periodically clipped grass is largely of fiber and other nitrogen-free substances. However this may affect the feeding value of the forage, one must not lose sight of the fact that the effects of clipping upon root growth and upon storage of reserves do govern over a period of years a perennial plant's ability to withstand adverse environmental conditions. This is brought out in the contrast between the stem counts in the garden experiment (table 2) and the greenhouse experiment (table 1). The general lack of vigor of the plants in the greenhouse clipping experiment which were also subjected to high temperatures and low light intensity lends stress to the point made by GRABER (4) that the productive capacity of grasses depends upon adequate supplies of available nutrients and moisture combined with favorable light and temperature conditions as well as upon adequate food reserves.

SOIL TEMPERATURE AND CLIPPING

In an attempt to determine the effects of soil temperature combined with clipping upon different types of brome grass, clone 4, a creeper from Washington; clone 11, a Parkland bunch type; clone 25, a southern type, with origin at Superior, Nebraska; and clone 26, a northern type with origin at Mandan, North Dakota, were selected for experimentation. The divisions of clones 4 and 11 were made by slicing uniform-sized root segments and accompanying stems from uprooted plants that had been established in the garden; those of clones 25 and 26 by slicing into uniform segments plants that had been established in 6-inch pots in the greenhouse. The root mass of the divisions of each clone was relatively uniform, but the accompanying stem numbers varied greatly. The divisions were planted in 8-inch glazed pots on August 24, 1946, without any removal of leaf tissue, and were watered when necessary. By September 17 the plants appeared well established and were subjected to soil temperatures of 20°, 26°, and 31° C. by means of the University of Chicago soil temperature tanks. Because of limited facilities only one division of each clone in the unclipped series was subjected to each temperature. For the clipped series, clones 25 and 26 were run singly and clones 4 and 11 in duplicate; they were clipped on October 2. All plants were harvested on November 12, and the dry weights of both roots and tops and the numbers of roots were determined.

Only clones 25 and 4 survived clipping at all soil temperatures. Clone 11 survived clipping at 20° and 26° C., clone 26 only at 20°. The survival of clone 25 and death of clones 26 and 11 at the higher temperatures was possibly related to latitude of origin. This would agree with the observations of NEWELL and KEIM (8) that southern strains were more tolerant of midsummer heat and drought than northern strains. It was quite evident that removal of photosynthetic tissue accompanied by the rapid respiratory rate induced by high root temperatures was very unfavorable for even the strains that survived.

The root systems developed at the lowest temperature were fibrous and much branched, while at the highest temperature few roots of the second and third order were in evidence. STUCKEY (10), working with colonial bent grass, also found maturation of roots to be accelerated by higher soil temperatures, with little branching in evidence.

Total root weight was inversely correlated with soil temperature in all four

clones (table 4), as was the weight of individual roots. The data reflect markedly the limiting effects of high soil temperature upon root growth in this species and also the limiting effect of clipping.

The total numbers of roots of the three clones of northern origin in the unclipped series were also inversely related to soil temperature. However, clone 25, of southern origin, showed the maximum root number at 26° in the unclipped series. In the clipped series the inverse relation to temperature held for all clones except no. 4, where the lowest number of roots was found at 26° C. With the exception of clone 4 at 20° C. and clone 25 at 31°, the numbers of roots were lower on all the clipped plants than on the unclipped plants, irrespective of soil temperature.

Although in general the numbers and dry weights of stems were inversely correlated with soil temperature (table 4), the effects were not so marked as in the roots. Since the underground parts of the stems were subjected directly to the different temperatures, the results do not differentiate the direct from the indirect effects of soil temperature upon the stems.

With the exception of clone 4 at 20° the number of stems of all clones decreased as an effect of clipping, with greater decrease at higher soil temperatures. In the unclipped series one northern clone (no. 11) decreased in live stem number at the highest soil temperature; and the percentage increase in the others was least at the highest temperature. In the southern clone (no. 25) the greatest increase was at 26°.

Total top weight in the unclipped series was inversely correlated with soil temperature in two of the northern clones (26 and 11). Clone 4 yielded most at 20° C., with a slightly greater yield at 31° than at 26°. The southern clone (no. 25) produced the greatest top weight at 26° and the least at 31° C.

In the clipped series the data do not include the weights of tops clipped on October 2. In all clipped plants the final yields at 20° C. exceeded those at 26° or 31°, although in clone 26 the yield at 26° was near that at 20°.

The weight per stem in the unclipped series was inversely correlated with soil temperature for clones 26 and 11 but directly so for clone 4. Clone 25 had the greatest weight per stem at 26° C. and the least at 20°. The surviving plants in the clipped series had the lightest stems at 20° C. with the exception of clone 4 (table 4). This tendency toward fewer but heavier stems in the clipped plants at higher soil temperatures could result from an inhibition in either the initiation or elongation of new tillers with the consequent utilization of available nutrients and reserves by the surviving stems.

A measure of the total yield in proportion to the initial size of the clonal divisions was derived by dividing the final dry top weight by the stem numbers on September 17. In the unclipped series this value in two of the northern clones was inversely correlated with temperature, while in clones 25 and 4 it was greatest at 26° C. Clone 4 showed the least yield at 31° C.

In the clipped series all clones produced less dry-weight yield of tops per root at the higher temperatures, except clone 25, in which it was highest at 26° C. In the unclipped series this value was inversely correlated with soil temperature except in clone 4, in which it was highest at 20° C. and lowest at 26°.

The top-weight/root-weight ratio and weight-per-stem/weight-per-root ratio were positively correlated with soil temperature. This trend in these ratios is

TABLE 4

EFFECTS OF SOIL TEMPERATURE AND CLIPPING

Soil temperature (° C)	Clone 25		Clone 26		Clone 4		Clone 11	
	Unclipped	Clipped	Unclipped	Clipped	Unclipped	Clipped	Unclipped	Clipped
	Dry wt. of roots (gm.)							
20	0.83	0.58	1.30	0.21	5.20	2.01	4.25	0.74
26	.79	.33	0.53	§	1.94	0.36	1.41	0.57
31	0.11	0.05	0.17	§	0.99	0.42	0.43	§
	Wt. per root (mg.)							
20	9	8	5	2	27	9	19	5
26	7	7	2	§	10	4	11	4
31	3	1	1	§	6	4	4	§
	No. of roots							
20	90	77	265	114	195	220	219	145
26	113	50	257	§	189	99	134	129
31	37	43	150	§	163	118	121	§
	Dry wt. of tops (gm.)							
20	2.19*	0.85†	3.81*	0.89†	13.73*	4.78†	15.47*	1.90†
26	2.46*	.71†	1.74*	§	9.68*	1.26†	7.80*	1.20†
31	0.64*	0.36†	0.71*	§	9.80*	1.41†	3.12*	§
	Wt. per stem (mg.)							
20	104*	57†	93*	81†	270*	150†	430*	130†
26	135*	71†	76*	§	320*	130†	340*	150†
31	128*	72†	65*	§	360*	180†	170*	§
	Relative no. of stems‡							
20	150	83	186	79	196	100	133	68
26	200	71	121	§	187	50	135	33
31	125	45	110	§	104	40	90	§
	Dry wt. of tops per root (mg.)							
20	24*	11†	14*	8†	70*	22†	71*	13†
26	22*	14†	7*	§	51*	13†	58*	9†
31	17*	8†	5*	§	60*	12†	26*	§

* Based on total top yield.

† Based on top yield produced after clipping on October 2.

‡ Number of stems on November 12 as percentage of number on September 17.

§ Dead.

TABLE 4—*Continued*

Soil temperature (° C)	Clone							
	25		26		4		11	
	Unclipped	Clipped	Unclipped	Clipped	Unclipped	Clipped	Unclipped	Clipped
	Total top wt./total root wt.							
20	2.6*	3.3*	2.9*	7.1*	2.6*	5.9*	3.6*	10.8*
26	3.1*	7.0*	3.3*	§	5.0*	13.0*	5.5*	15.2*
31	5.8*	13.2*	4.2*	§	9.9*	25.3*	7.2*	§
	Wt. per stem/wt. per root							
20	11	.....	19	.....	10	.....	22	.....
26	19	.....	36	.....	32	.....	32	.....
31	43	.....	59	.....	60	.....	47	.....

related primarily to the strong inverse correlation of root weights, both total and individual, with soil temperature. The adverse effect of clipping upon root growth is indicated by a comparison of the top-weight/root-weight ratios in the clipped series with the unclipped series (table 4).

In this experiment soil temperature exerted relatively greater control over root development than it did upon growth of the tops. Under the conditions of this experiment, adequate moisture was supplied to all plants, and the tops probably did not suffer water deficiency because of inadequate root systems, even at the highest soil temperature. Under field conditions, however, such limitation on root growth with the advent of warm soil temperatures accompanied by lack of moisture in the upper layers of soil would not be conducive to the survival of this species in competition with better adapted species, especially if grazed or mowed simultaneously. The lack of vigor observed in the plants clipped during the summer of 1946 in the greenhouse clipping experiment (table 1) was no doubt in part caused by the removal of photosynthetic tissue with consequent reduction in the synthesis of carbohydrate at the same time that the roots were subjected to high daytime soil temperatures bringing about an increased rate of respiration.

Shading

Three divisions of the same twenty-three clones used in the greenhouse clipping experiment were used in an experiment on shade tolerance set up at the same time. The plants grew in the full natural light of the greenhouse until June 30, 1946, and were then apparently well established. They were then clipped back to a height of 2 inches but were not clipped thereafter. They were watered as necessary, and each plant was given 192 ml. of nitrate solution on May 4, 1946, on September 5, 1946, and on April 3, 1947. Series C remained in the full greenhouse light; series 1 was shaded by a single thickness of unbleached sheeting of a type used for shade-grown tobacco,

and series 2 by a double thickness. The average values of approximately simultaneous light readings made with a Weston Sunlight Meter during the course of this experiment were: outside, 5233 foot-candles; greenhouse, 2417 f.-c.; under one thickness of sheeting, 731 f.-c.; under two thicknesses of sheeting, 251 f.-c., or a ratio of approximately 100:46:14:5.

By September 27 only four clones had survived in series 2. At the termination of the experiment on June 2, 1947, all series 2 plants had died, but ten clones were still surviving in series 1. The clones which survived longest under the double thickness of cloth were among those surviving under the single thickness almost 8 months later. Six of the plants in series C had died by the termination of the experiment. Two of these were clones that survived in series 1, though not in series 2, and the other four died at about the same time as they did in series 1. Although the light intensity in the greenhouse was less than half that in full sunlight, some factor or factors other than low light intensity must have been the cause of death in most series C plants, as all but one died during the summer and fall of 1946. The same conditions of high air temperatures and infestation with greenhouse pests enumerated in the greenhouse clipping experiment prevailed during the course of this experiment and made growing conditions for all series decidedly adverse. The initial clipping and consequent limitation of photosynthetic tissue in all series, coupled with high air and soil temperatures and lowered light intensity, were quite favorable for a high rate of respiration and limited production of photosynthate.

WATKINS (11) found in a field experiment with *B. inermis* that a reduction of light intensity to 300–800 foot-candles brought about a decrease in the number of shoots, number of rhizomes, number of fertile shoots, and dry weight of all plant parts, and an increase in the number of elongated internodes and in height of the plant. The effects of shading in the present experiment suggest inherent differences in the shade tolerance of the different clones. There seemed to be little correlation of shade tolerance with latitude of origin, since one of the surviving clones in series 2 had its origin in Washington, Kansas, and one in Saskatchewan. Neither did there seem to be any correlation with growth habit, as clones that were characteristically creepers as well as those that were bunch types were found among both the surviving and the dead plants in all series.

Under field conditions, where the plants grow close together and competition for light as well as for mineral nutrients and water is keen, those individuals that were not tolerant of shading would most certainly be crowded out. Ability to withstand shading, although of selection value in a field of pure brome grass, would be even more important in mixed fields of brome and alfalfa in which the presence of shade-tolerant strains might well determine the survival of brome grass in the mixture.

SOIL VOLUME

Equal divisions of four different clones which had been growing in the garden were planted in containers of seven different types and sizes on August 29, 1946, in an attempt to find what effect soil volume might have upon vegetative growth under greenhouse conditions. Tops and roots were trimmed as little as possible in transplanting, and all conditions under which the plants were grown were similar except the volume of soil in the different containers, the shape of the

containers, and the material of which the containers were constructed (table 5). Although each plant was given water as seemed to be necessary, rapid evaporation through the walls of the smaller unglazed clay pots probably removed the available water from the outer part of the soil mass so rapidly that the plants in these containers did not have sufficient moisture available to them at all times over the surface of the soil, with some emerging from the space between the soil and the edge of the container. Root distribution was somewhat less uniform and was different for different clones, though in general the roots were well distributed throughout the soil in the larger containers and tended to be concentrated around the bottom and sides of the smaller ones. The roots of all clones in the

TABLE 5

RESULTS IN SOIL-VOLUME EXPERIMENT

Container	Relative soil volume	Original stem count	Final stem count	Final stem count as % of original	Total top wt. (gm.)	Top yield per original stem (gm.)	Av. wt. of each stem (gm.)	Total root no.	Total root wt. (gm.)	Av. wt. of each root (mg.)	T/R	Roots per stem	Top yield per root (mg.)	Wt. per stem/wt. per root
12-in. square wooden box.	12	22	150	682	35.13	1.6	0.23	754	5.42	7.2	6.5	5.0	4.7	32
12-in., deep clay pot*...	10	26	139	534	38.92	1.5	.28	795	8.98	11.3	4.3	5.7	4.9	25
8-inch, glazed crock†.....	4	28	133	475	38.56	1.4	.29	880	8.15	9.3	4.7	6.6	4.4	31
10-in., deep clay pot*...	5	26	114	439	28.93	1.1	.25	590	8.11	13.7	3.6	5.2	4.9	18
10-in., shallow clay pot*...	2	27	85	315	13.66	0.51	.16	457	3.74	8.2	3.7	5.4	3.0	20
8-in., deep clay pot*.....	2	26	79	304	18.98	0.73	.24	491	7.42	15.1	2.6	6.2	3.9	16
6-in., deep clay pot*.....	1	25	77	308	14.73	0.59	0.19	421	5.68	13.5	2.6	5.5	3.5	14

* Standard greenhouse pots, sides tapering to base. † 10 inches deep, sides parallel.

for maximum growth. Nitrate solution was applied to all plants on October 15 and November 27 at the rate of 100 ml. per unit of soil volume (volume of 6-inch clay pot taken as unity). Since the applications had no apparent effect upon the vigor of the plants, nitrogen deficiency which could be so corrected was probably not a limiting factor in any container. The plants were harvested on December 10, 1946, and data were obtained on the oven-dry weights of tops and roots and on root numbers (table 5).

In all containers the emergent stems at final harvest were scattered at random 8-inch glazed pots were well distributed through the soil.

The results in table 5 are an average of the values for the four clones at final harvest. With the exception of the 8-inch glazed container, in which results were similar to those in the 12-inch unglazed clay pot, the columns express the results in order of relative volume of container. The final stem count was positively correlated with soil volume as was the percentage increase in stem number. The dry top weight was much lower in the smaller soil volumes, but the trend was not so regular as with stem numbers.

The top yield per original stem was also somewhat proportional to soil volume, although it was least in the 10-inch shallow clay pot. The weight per final stem did not vary so significantly, though it was highest in the 8-inch glazed pot and lowest in the 10-inch shallow clay pot.

Total root number showed no regular relation to soil volume but was greater in the larger containers and was largest in the 8-inch glazed pot. The second largest total root weight was also produced in the 8-inch glazed pot, which had a relatively low soil volume. Dry root weight in general showed no correlation with soil volume, nor did the weight of each root, but the top-weight/root-weight ratio in general was somewhat proportional to soil volume because of the correlation of top weights with the latter.

The number of roots per stem was fairly constant, regardless of the size of the container. The top yield per root and the weight-per-stem/weight-per-root ratio trended with soil volume.

In summary, in contrast to the relative lack of correlation between root data and soil volume, the tops of the plants showed a direct correlation of total stem numbers and total weight with soil volume (table 5). This positive correlation is also primarily responsible for the direct correlation of soil volume with the top-weight/root-weight ratio, the weight-per-stem/weight-per-root ratio, the top yield per root, and the top yield per original stem.

In all the unglazed clay containers the top growth was in direct proportion to the size of container, but in the glazed crock the top growth far exceeded that attained in a slightly larger unglazed clay pot and was almost equivalent to that attained in a clay pot two and a half times as large. It would thus seem advisable to use glazed pots wherever possible in preference to unglazed clay, and, if unglazed clay pots must be employed, to use the largest ones possible. The limitation placed upon top growth by the 8-inch clay pots in this experiment would seem to indicate that container size was a limiting factor in both the greenhouse clipping experiment and the shading experiment.

Discussion

Based upon the results of these experiments, certain general recommendations can be made with respect to the culture of *B. inermis* under greenhouse conditions. Since high soil temperatures had very adverse effects upon root growth, especially when photosynthesis was curtailed either by removal of leaf tissue or by shading, it would seem advisable to use large pots and to surround them with sand, peat moss, or some other insulating substance to reduce to a minimum the heating effect upon the soil of the extreme daytime air temperatures reached in summer. The results with containers of different kinds indicated that pot size, shape, and type might be an unforeseen limiting factor in many greenhouse experiments. A deep glazed pot of the largest size that is practicable with available space should be used. When it is necessary to carry greenhouse plants that have been weakened by experimental treatment through natural low light intensities comparable to those in winter in Chicago, the use of supplementary artificial illumination during the normal photoperiod would seem advisable.

However, it should be pointed out that, despite the relatively poor growing conditions in the greenhouse, the effects of two, one, and no clippings upon plants in the greenhouse, as measured by the percentage differences in top weights and stem counts in the three series, were

closely comparable to the effects of similar treatments upon plants in the garden, even though absolute values for these criteria of growth were widely different between greenhouse and garden plants.

Whereas a range of soil temperatures and clipping intensities showed stronger correlation with root growth than with top growth, soil volume exerted its effects most markedly upon the tops.

Summary

1. Vegetative responses of diverse plants of smooth brome grass (*Bromus inermis* Leyss.) to variations in soil temperature, container size, clipping intensity, and shading were investigated in the greenhouses and garden of the University of Chicago with plants derived from both clonal divisions and seed from diverse places of origin. The responses of the individual plants were measured in one or more of the following ways: stem count; total oven-dry top weight; root count; and total oven-dry root weight.

2. The effects of clipping upon plants of diverse origins were studied in three separate experiments: clipping in greenhouse (seventeen clones); clipping in garden (twenty-two clones); and clipping seedlings in greenhouse (three strains of diverse origin). Three series were run in the experiments on clones started in April, 1946: series C, control; series 1, clipped once; and series 2, clipped twice in the 1946 growing season. Throughout the course of the experiments with clonal materials, which were terminated in June, 1947, stem counts were made and dry weights of clipped tops determined. Dry weights of tops and roots were determined for the seedlings. At the final harvest dry weights of tops and roots and the number of stems were inversely proportional to frequency of clipping. In the greenhouse experiment, where light and temperature as well as container size were limiting factors, the weight per stem did not seem to be affected by clipping, but in the garden experiment it was inversely correlated with frequency of clipping. It was further found that clipping was more adverse in its effects upon root growth than upon top growth and that the effect upon both carried over into the season following clipping.

3. Divisions of four clones were grown at soil temperatures of 20°, 26°, and 31° C. Greatest total top weight was produced at 20° C. in three clones of northern origin and at 26° in one clone of southern origin. At all temperatures the increase in number of stems was less in the clipped series than in the unclipped, and the increase was inversely proportional to the soil temperature. One northern clone did not survive clipping at 31° C., and another died at both the 31° and the 26° soil temperatures. The weight of single roots and the total root weight in each clone showed a much stronger inverse correlation with soil temperature than did total top weight.

4. Three divisions of twenty-three different clones were brought into the greenhouse from the garden and planted in 8-inch clay pots in late April, 1946. One series was placed on a well-lighted bench in the greenhouse, another under a single layer of sheeting, and the third was placed under a double layer of sheeting. Relative light intensities were: outside, 100; greenhouse, 46; one layer of cloth, 14; two layers, 5. At the lowest intensity only four clones survived until the fall of 1946. In the spring of 1947, when the experiment was terminated, ten clones were still alive under the single layer of sheeting, seventeen were still alive in the control series, but none had survived in the most shaded series.

5. Divisions of four clones were grown in unglazed clay pots of five different sizes, in 8-inch glazed pots, and in 12-inch-square wooden boxes. With the exception of the plants in the glazed pots, the final stem count, oven-dry top yield, top yield per original stem, total root number, top-weight/root-weight ratio, top yield per root, and ratio of weight per stem/weight per root were directly proportional to the size of the container. In the glazed pots the plants were as vigorous as in clay pots two and a half times larger.

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FLOWERING OF SMOOTH BROME GRASS UNDER CERTAIN ENVIRONMENTAL CONDITIONS[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 588

HAROLD J. F. GALL

Introduction

The increasing importance of smooth brome grass (*Bromus inermis* Leyss.) as a forage crop and in reseeding denuded ranges has led to the necessity for more accurate selection of types adapted to a wide range of latitudes and climatic conditions and to a program of developing improved strains. A knowledge of the conditions necessary for vigorous production of panicles and the setting and maturing of seed, both in the field and under greenhouse conditions, is needed for the efficient carrying-out of such work. In this study a series of clones of brome grass, representing various strains, was examined with regard to these requirements, with especial attention to the production of viable seed, and to determine to some extent the factors involved in the initiation of floral primordia and their subsequent development. The experiments were carried on at the University of Chicago during 1946 and 1947.

The wide range of variation within the species has been recognized for many years. WALDRON (13), in a study of several thousand seedlings of brome grass in North Dakota, found much variation in physiological and morphological characters. He noted a range from a strongly "fertilclinous" condition to a strongly "sterilclinous" one and a similar range in tillering habits. NEWELL and KEIM (7) have reviewed the history of the species in the United States. In field trials at Lincoln, Nebraska, they noted distinct differences between plants originating from northern sources and those from Nebraska and Kansas in vegetative growth and panicle production. Wide differences among strains in their adaptation to this region were apparent. EVANS and WILSIE (3), in greenhouse experiments, found that three clones—early, midseason, and late-maturing, respectively—responded differently to changes in daylength, temperature, and level of fertility. WATKINS (14), using seed of a commercial strain from Saskatchewan, concluded that brome grass was an indeterminate species photoperiodically, in the sense of GARNER (5), in that most fertile shoots were produced on natural daylength. ALLARD and EVANS (1), however, after finding no flowering on photoperiods of 10–13 hours and more vigorous flowering with longer photoperiods beyond 13 hours, assigned the species to the long-day class.

Material and methods

To insure genetic uniformity clonal material was used in all experiments. The clones available at the time of experimentation provided a range of morphological habit and place of origin.[2] OM-1 traces back to a plant selected for selfing in a bulk lot of the Canadian variety, Parkland. OM-2 goes back to a Nebraska

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[2] Material of all clones and data on their origin were supplied by Dr. ETLAR L. NIELSEN, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils and Agricultural Engineering, United States Department of Agriculture.

strain, B-4. OM-3 is a selfed line out of PI 115331 from Leningrad, Russia. Cl 3 represents the isolation of the better plants from PI 115334 from Leningrad. Cl 8 is an isolation from FC 22435 (original source not known at the time of writing). Cl 9 is an isolation from PI 101647, received from Manchuria. The origin of Cl 19 and 65 is not known. Cl 23, 27, 31, and 84 are all selected plants from bulk lots of Parkland seed. 914-1 is from the first inbred generation of a plant of the Parkland variety. The plants used represent the species over a latitudinal range of approximately 20°.

Equal divisions of each clone were made from plants previously established in the experimental garden and were planted in 8-inch unglazed clay pots. The soil used was a heavy clay loam of prairie origin, lightened with a mixture of sand and fine gravel. It undoubtedly contained a supply of mineral nutrients adequate to prevent deficiencies during the course of the experiments. Ten days after potting, all plants had shown vigorous growth. They were watered as often as was necessary to keep the soil constantly moist.

Supplementary light in experiments I and II was supplied for all except two series by banks of twelve 40-watt fluorescent lamps of the "Daylight" type mounted $2\frac{1}{2}$ inches apart on a white-enameled reflecting board. These gave a light intensity of about 800 foot-candles at a distance of 10 inches below. Lights were raised periodically as the plants grew. Pots containing plants of slower growth were elevated individually to keep the general foliage level of a series fairly uniform.

In experiment I in 1946, two of the series, designated 17M and 15M, were given supplementary light from 200-watt incandescent-filament lamps mounted in individual reflectors. Light intensity at plant level was about 150 foot-candles. These two series were placed on movable trucks and received natural daylight for 9 hours in the open greenhouse and artificial light for 8 and 6 hours, respectively, after being moved into ventilated lightproof sheds.

Three of the series in 1946 were grown on a greenhouse bench with the banks of fluorescent lamps suspended above them, and the lights were kept burning throughout the light period. Black cloths, coated white on the inside surface, were hung from each reflector to inclose the plants during the dark period. These were opened on the side to the south during the day, and on sunny days the reflectors and cloths were raised to allow maximum and equal illumination of all plants. Temperatures inside the cloths were found to vary considerably, long photoperiods resulting in higher temperatures; consequently, later experiments were run in the sheds or in the open greenhouse without the inclosing cloths.

Flowering response was evaluated by noting the first exsertion of the inflorescence from the ensheathing leaves and by the later development and vigor of panicles. In some cases the status of the apical meristem in respect to vegetative or reproductive condition was determined by dissection and microscopic examination. Vegetative response was evaluated mainly by maximum height of plants, number of new tillers produced, number of tillers showing elongation, and dry weights of roots and tops at final harvest.

Experimentation

EXPERIMENT I.—Twelve dormant plants were brought into the greenhouse on January 10, 1946, and on January 11 small pieces of rhizomes, fairly uniform

as to size and condition of growing points, were planted in individual pots. They rapidly resumed growth, and on January 22 one pot of each clone was placed under each of six treatments. Three photoperiods—17, 15, and 13 hours—were used with fluorescent light, and two photoperiods—17 and 15 hours—with incandescent-filament light. A series (*N*) was also run on natural daylength and light intensity.

Air temperatures given in table 1 represent averages of measurements over the course of the experiment, which was terminated, except for the *N* series, on April 18, 1946.

TABLE 1

AIR TEMPERATURE (IN °F.) AT PLANT LEVEL. EXPERIMENT I

Series	Av. min.	Av. max.	Range	Mean
17-hour	68.6	85.3	69–90	77.0
15	66.5	84.8	62–89	75.7
13	66.2	82.3	62–90	74.3
17M	63.3	75.2	55–79	69.3
15M	58.3	69.8	51–74	64.1
N	63.7	70.8	58–90	67.3

Flowering response, as based on the date on which the first inflorescence was exserted in each plant, is shown in table 2. Anthesis followed in approximately 14 days in all cases, with the exception of the weak or sterile panicles. Six of the twelve clones flowered on 17-hour photoperiod; four of these six flowered also on 15-hour photoperiod, though less vigorously and from 5 to 26 days later. Five of the six flowered also on 13-hour photoperiod, in addition to two clones that flowered only on this light period. Inflorescences on 13-hour plants were at best of only fair vigor in comparison with 17-hour plants. Three of the controls on natural daylength flowered, though poorly and from 34 to 44 days later than on 17-hour photoperiod.

Differences in vigor of growth and flowering between the 17-hour series under fluorescent and under incandescent-filament light were marked. Five plants of the latter series flowered, producing rather poor panicles from 4 to 21 days later than the plants of the same clones under the higher light intensity of the fluorescent lamps. The 15M series was inferior in all respects to any of the others. Three plants exserted inflorescences in 46 days, but they were of insufficient vigor to reach anthesis. It is emphasized that the 17M and 15M series received only natural daylight for 9 hours of the photoperiod, while the series under fluorescent light received artificial light continuously during the photoperiod. Considering the many very cloudy days of the Chicago winter as well as the relatively low intensity of the supplementary incandescent-filament light, the total illumination of the M series was undoubtedly below the minimum required for normal growth. The lower range of temperatures (table 1) to which these plants

TABLE 2

NUMBER OF DAYS UNTIL FLOWERING AFTER BEGINNING OF PHOTOPERIODIC TREATMENTS ON JANUARY 22

Clone		Series					
		17	15	13	*N*	17M	15M
OM-	1			68			
	2						
	3	23	28	37*		33*	47*
Cl	3	24		30	68*	28	46*
	8						
	9	17	33			38	
	19	28		54		38	
	23			56*			
	27	22	48*	38*	56*	28*	46*
	31						
	65	21	28	28*	56*		
	84						

* Poor and sterile panicle.

were exposed during the period of experimentation was probably another factor contributory to their lack of vigor.

No seed was matured by any plants, although the panicles produced by the 17-hour plants under fluorescent light appeared to be vigorous and the pollen under microscopic examination to be normal. It was thought probable that the high temperatures resulting from inclosing the plants and lights with heavy lightproof cloth were responsible for this failure. Further experiments were modified to provide better control of this factor.

In vegetative response there were marked differences among treatments under fluorescent lights. The twelve clones, at the time of harvest on April 18, had produced totals of 74, 56, and 26 elongated tillers on 17-, 15-, and 13-hour photoperiods, respectively. Numbers of unelongated tillers were inversely correlated with photoperiod, with totals of 97, 146, and 218. Total numbers of tillers —171, 202, 244—were also inversely correlated with length of the light period. Maximum height was measured from the soil surface to the tip of the longest leaf or inflorescence and was directly correlated with length of photoperiod for most clones, the averages being 66.6, 55.6, and 50.0 cm., respectively. These differences were clearly reflected in the general aspect of the plants (figs. 1–3).

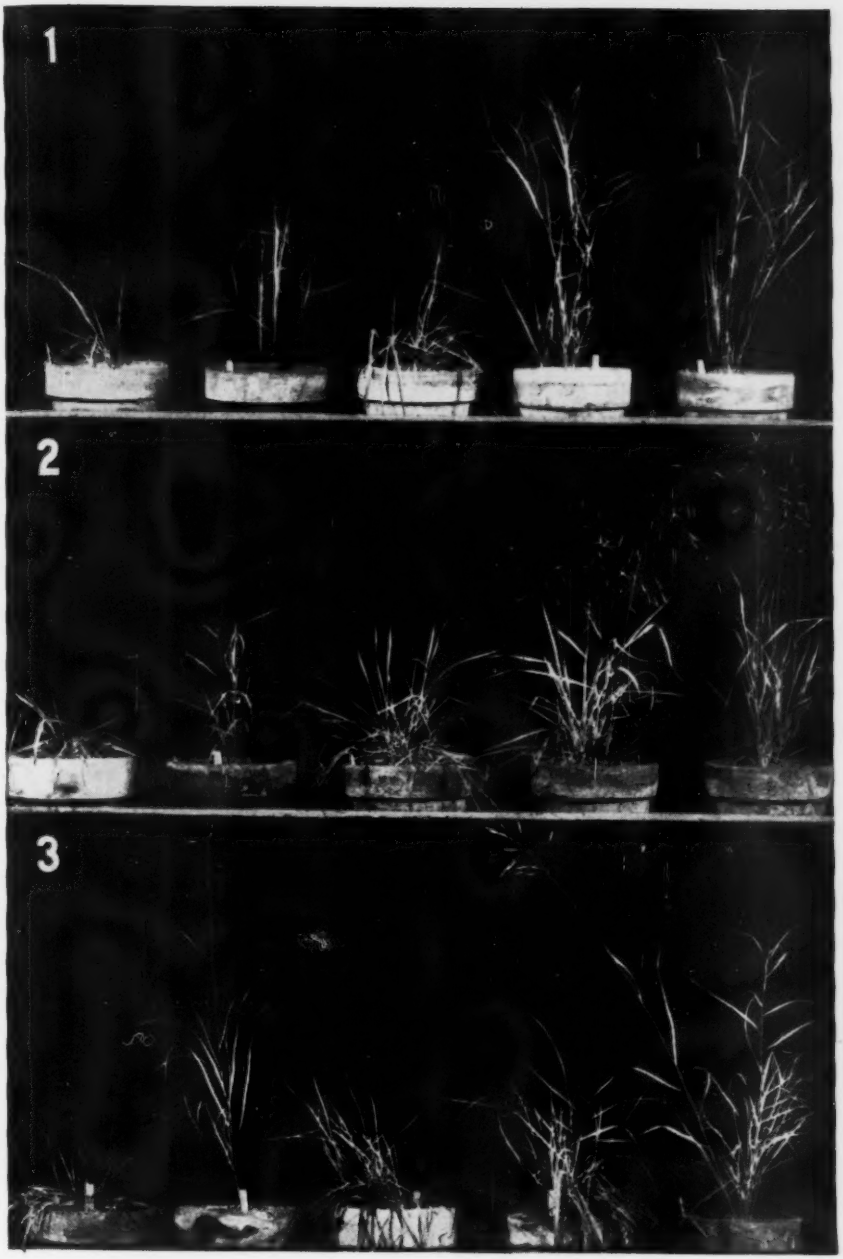
Comparable data for the 17M and 15M series, respectively, are: elongated tillers, 44 and 15; unelongated tillers, 37 and 121; total tillers, 81 and 136. They show clearly the limited growth made under low light intensity and average low temperature in these series in contrast with those under fluorescent lights, and also the effect of the longer photoperiod in inducing elongation of tillers.

Total numbers of rhizomes for the twelve clones showed no apparent influence of photoperiod but a strong influence of light intensity and temperature. The 17-, 15-, and 13-hour plants produced a total of 40, 39, and 41 rhizomes, respectively, while the 17M and 15M series produced only 5 and 6.

EXPERIMENT II.—In this experiment plants were brought into the greenhouse from the garden at two different times, on August 31, 1946, and again on December 27, to test the effect of exposure to low temperatures and of long establishment in the pots on floral initiation and emergence. Six of the clones used in experiment I were used. Of these, Cl 9 and Cl 27 had flowered readily on long photoperiod, OM-1 and Cl 23 had flowered only on a short light period, and OM-2 and Cl 84 had not flowered on any treatment (table 2). Four divisions of each were made on each date. Large pieces of entire plants were used for each division rather than single rhizomes.

After potting, the August plants were trimmed of dead parts and old sterile culms but otherwise left intact. They were kept on natural daylength in the greenhouse until differential treatment was begun on January 7, 1947. During this period they were given several hours of artificial light on very cloudy days to prevent too great loss of vigor; this was supplied without extending the photoperiod. Growth during September to January was limited, and most of the plants had declined perceptibly in vigor by the time treatments were begun. Food reserves for further growth were thus probably low, and the response of these plants to photoperiodic treatments in contrast to that of the December plants may have been conditioned by this fact as well as by lack of pre-chilling.

On January 7, two series, each including August and December plants, were



FIGS. 1–3.—Clonal divisions of smooth brome grass grown from single rhizomes brought into greenhouse on January 10, 1946. After January 21 they were subjected to (*left to right*): Chicago natural daylength and light intensity (series *N*); 17 hours of relatively low light intensity (series 17M); 13, 15, and 17 hours of relatively high light intensity (fluorescent light). Fig. 1, Cl 84; fig. 2, OM-3; fig. 3, Cl 27. (Photographed April 16, 1946.)

placed on movable trucks in lightproof sheds. Banks of twelve 40-watt fluorescent lamps as described above were used to provide 18- and 13-hour photoperiods. On sunny days the trucks were moved out of the sheds to provide maximum light intensity throughout the light period. In addition to a series (*N*) on natural daylength and light intensity in another greenhouse room, an additional 18-hour series (18C) was run under fluorescent lights in still another greenhouse room as a check on conditions in the sheds.

TABLE 3

AIR TEMPERATURES (IN ° F.) AT PLANT LEVEL. EXPERIMENT II

Series	Av. min.	Av. max.	Range	Mean
18-hour	67.0	74.3	63–82	70.7
18C	66.8	74.3	57–87	70.6
13	63.8	72.6	55–79	68.2
N	66.6	74.1	59–88	70.4

Temperature data (table 3) indicate a much better control of this factor than in 1946.

Flowering data are given in table 4. Of the plants brought in on December 27, all clones flowered on 18-hour photoperiod within 26 days. Anthesis followed about 2 weeks later. There was good agreement between the 18 and 18C series. One clone, Cl 9, flowered on 13-hour photoperiod, 20 days after first flowering on long photoperiod. Series 18, 18C, and 13 were terminated on March 29. None of the control (*N*) plants had flowered by June 1, although the same clones then outdoors had done so, as they also did when brought into the greenhouse on April 14 and left on natural daylength. This contrast in plants differentiy treated on natural daylength is further evidence that exhaustion of carbohydrate reserves may be a factor involved in failure to flower under greenhouse conditions.

Of the August plants, all on 18-hour photoperiod showed marked tiller elongation (figs. 4–6), but no inflorescences were produced. Stem tips of these plants were examined at the close of the experiment on March 29, and all were found to be vegetative.

Tiller counts were made of all plants on January 23 and again on March 26 (table 5). For the August plants, averages of increase in tiller number in the 2 months for the two 18-hour series were lower for each clone than for the 13-hour series. Tiller counts for the December plants showed less correlation with length of photoperiod. This may perhaps be attributed to the shorter time available to these plants for becoming established in the pots before being placed under the lights. Relatively, the *N* series produced most new tillers and the 13-hour series the least.

TABLE 4

NUMBER OF DAYS UNTIL FLOWERING AFTER BEGINNING OF PHOTOPERIODIC TREATMENTS ON JANUARY 7. DECEMBER PLANTS

Clones		Series			
		18	18C	13	*N*
OM-	1	23	23	.....	.....
	2	26	26	.....	.....
Cl	9	21	20	41	.....
	23	26	23	.....	.....
	27	23	23	.....	.....
	84	22	30	.....	.....

Average maximum heights on March 21 for series 18, 18C, 13, and *N*, respectively, were: August plants, 69, 74, 43, and 34 cm.; December plants, 79, 83, 55, and 38 cm. Height was thus greatest in the 18-hour plants and least in the *N* series. December plants were taller than August plants on 18-hour photoperiod, as in most cases the flowering culms overtopped the vegetative tillers of the latter.

Dry weights of tops (table 6) of the August plants were greatest in the 18-hour series; of roots, in the 13-hour series. The plants were cut off at the soil surface, and all below-ground parts were evaluated together as roots with no attempt to distinguish rhizomes and stem bases.

The December plants showed trends similar to those of the August plants in regard to dry weights. Their absolute weights were somewhat greater; since the initial divisions in August and December were approximately equal, this may reflect the failure of the August plants to grow vigorously after being brought into the greenhouse.

Four other clones that were still available, namely, Cl 8, 19, 31, and 65, and a new plant, 914-1, were also brought in during December and were subjected to somewhat similar treatments as the other six. Results were substantially in agreement. Clone 914-1 was the only plant of this group to bloom on 13-hour photoperiod after 39 days. It also bloomed on the 18-hour photoperiod after 21 days.

TABLE 5

PERCENTAGE INCREASE IN NUMBER OF TILLERS PER PLANT, JANUARY 23–MARCH 26

PLANTS	SERIES			
	18	18C	13	N
August	13.7	36.2	42.8	77.8
December	27.7	38.6	27.1	68.6

Failure to obtain the production of seed in experiment I was a principal reason for repeating the work in a revised form. In 1947, panicles of different clones were shaken together when in anthesis to insure pollination, since the species has a high degree of self-sterility (2). Artificial light was supplied until it was evident that the panicles were mature. Under these conditions, most of the 18-hour plants which had flowered also formed seed which produced vigorous seedlings when planted.

EXPERIMENT III.—An important point in regard to the flowering of brome grass to be determined was the time of initiation of observable floral primordia in the Chicago area. To ascertain this, stem tips of plants of the eleven clones still in the garden in 1947 were dissected out periodically during the spring after growth had been resumed. Floral primordia were first discovered on Cl 9 on April 8, on OM-1, Cl 27, Cl 84, and 914-1 on April 9, on Cl 23 on April 10, and on OM-2 on April 14. In all cases the reproductive growing points were located at or near the ground level in unelongated shoots 20 cm. or more in height. The spring of 1947 was abnormally cold, and these dates may be considerably later than the average time of initiation.

TABLE 6

DRY WEIGHTS (GM.) OF PLANTS HARVESTED MARCH 29. AVERAGES ONLY

PLANTS	18-HOUR		13-HOUR	
	Tops	Roots	Tops	Roots
August	8.83	7.49	6.88	8.97
December	10.30	9.19	6.09	9.56

To determine to some extent the conditions necessary for the further development and elongation of the inflorescences, plants of these seven clones were brought in on April 14, potted, and put on 13-hour photoperiod, receiving 9 hours of natural daylight in the open greenhouse and 4 hours of supplementary light supplied by three 30-watt fluores-

FIGS. 4, 5.—Clonal divisions of smooth brome grass grown after January 6, 1947, on photoperiods of 13 hours (*left pair*) and 18 hours (*right pair*). Left-hand plant of each pair was brought into greenhouse on August 31, 1946; right-hand plant of each pair, on December 27. Fig. 4, OM-1; fig. 5, OM-2. Initial clonal divisions were much larger than for plants shown in figs. 1–3. (Photographed March 28, 1947.)

cent lamps in the shed. On the basis of the samples dissected, the majority of the larger tillers on all clones were then in the reproductive state. By May 12 clone 914-1 had produced several very depauperate inflorescences, and, by May 18, OM-1, Cl 9, and Cl 84 had produced completely barren elongated culms. On June 3 the remaining plants of this group that none of the tillers on the section brought in were reproductive on April 14.

These observations are in contrast to the behavior of divisions of clones Cl 23 and 914-1 also brought into the greenhouse on April 14 and allowed to grow under the increasing natural photoperiods. Tillers of these, dissected on April

FIG. 6.—As in figs. 4, 5. Cl 9

on 13-hour photoperiod were examined by dissection. Cl 23 had several tillers with panicles well developed and nearing exsertion, but the branchlets of the panicles were brown and withered except for the lowermost two or three, which were still green. About six internodes had elongated in each tiller examined. Cl 27 was in a similar condition but with inflorescences less developed. OM-2 showed only vegetative stem tips. This was a very small plant because of a lack of material in the garden, and it is possible 23, were found to have inflorescences up to 11 mm. long with branches well developed. One plant flowered on May 2, with normal panicles, and all were in bloom by May 14. This is in sharp contrast to the failure of the *N* series of experiment II to flower on the even longer photoperiods of late May and early June. It is possible that the *N* plants required a longer period of chilling in order to flower, comparable to that received by the plants brought in on April 14. It is more probable, however, that the subjec-

tion of the December *N* series to the adverse effects of 3 months of dark and short but relatively warm winter days in the greenhouse was more significant than the amount of chilling, in view of the excellent flowering of the December plants on 18-hour photoperiod after the same amount of chilling. The failure of the August plants to flower on 18-hour photoperiod may also be attributed partly to the adverse effects of too long a period in the greenhouse in small pots with short photoperiods and low light intensity.

Discussion

The pronounced effect of the longer photoperiods in promoting culm elongation and, under some conditions, the production of panicles in brome grass is in agreement with the results of most other investigators. The differences in the amount of flowering and in the time required for flowering between the plants on long photoperiods and those on natural daylength in winter were marked.

That the effect of long photoperiods is not primarily or perhaps not at all responsible for the initiation of inflorescences is indicated. Attainment of the physiological condition resulting in the formation of floral initials as the first morphological sign of the reproductive state is with little doubt caused by a complex of factors. The interrelations of temperature with photoperiod have been examined for a number of species of plants (10, 15). In the case of sugar beets, it has been found difficult to distinguish the roles of the two factors, and induction of flowers under the influence of prolonged low temperatures and long photoperiods is tentatively regarded as a single process (9). In brome grass the importance of chilling in the process is probably marked in many individuals in the species, even though in the clonal material used by EVANS and WILSIE (3) some of the plants brought in during October flowered well under certain conditions. The degree of chilling to which they had been subjected was unspecified. The need for chilling for floral initiation has been reported for other species of grasses. Some unspecified American work on cocksfoot is reported by WHYTE (15). Plants grown for 6 months under a 16-hour photoperiod in a greenhouse produced no heads; preconditioning by growing on short photoperiods and low temperature for 2–3 months resulted in flowering after removal to the 16-hour day. WHYTE also reported (15) that a late-flowering strain of perennial ryegrass was induced to flower early after exposure to winter conditions in Wales followed by transfer to continuous light plus higher temperatures.

Pre-chilling, however, is not invariably essential to flowering in brome grass. On July 1, 1946, seven clones were observed in anthesis in the greenhouse, including three used in the present work—Cl 9, 65, and 84. These plants had been in the warm greenhouse since June, 1945. This plant of Cl 9, still in the greenhouse, flowered again in 1947, on June 20. The August plant of the same clone in the *N* series of experiment II also was in flower on June 20. The internal balance or possible specific substances necessary to cause the change from the vegetative to the reproductive state apparently can be reached or formed through more than one complex of causes, in which temperature, light, age of plant, nutrition, genetic factors, and others, may be operative at various levels to produce the same end result.

The fact that floral primordia are formed by early April on brome grass in the field at Chicago indicates that a long

photoperiod is not required for initiation during the season of flowering, whatever may be its role in the previous season. KNOBLOCH (6) observed that on May 1 at Ames, Iowa, inflorescences are found near the ground level and are 1–1.5 cm. long. He stated that panicle primordia are initiated only in the year of anthesis. In northern Ohio the growing points of many perennial grasses remain vegetative during late summer and fall; floral primordia become visible in April or very early in May of the following year (4). WHYTE (15) concluded that it appears in England that "although grasses such as cocksfoot and ryegrass may be ripe-to-flower during the winter months (with no external evidence of this condition), the flower primordia are not laid down until March of the following year."

Long day, then, if it is an essential factor in floral initiation in brome grass, must apparently affect the plant in one growing season and the physiological effect be carried over to the next. It is not difficult to agree with SHARMAN, as quoted by WHYTE (15), who doubted whether daylength is of importance in the change from the vegetative to the reproductive state in perennial grasses such as ryegrass or sweet vernal.

Normal development and elongation of the culms and of the panicle initials, on the other hand, seem, in brome grass, to be definitely related to certain photoperiods. This is evidenced both by its flowering on natural daylength as the season advances and by the experimental results. It is significant that the clones referred to above as flowering without pre-chilling did so only after the days had lengthened, though temperature conditions did not preclude flowering at any time of year. Also, when plants with floral primordia already present were placed on 13-hour photoperiods, exsertion of the inflorescences was effectively inhibited in three clones, with only barren or depauperate panicles produced in the four others tested. The degenerating state of the inhibited inflorescences was similar to that described by TINCKER (11), who found that plants of early timothy on 6-, 9-, and 12-hour photoperiods all produced tillers containing young flowers checked in development and dying off. Sweet-scented vernal grass, prevented from flowering by 6-hour photoperiod, had floral primordia consisting of minute outer glumes inclosing a group of meristematic cells. In the Gramineae in general, he concluded, it is possible for the primordia (rachis and glumes) to be laid down and development arrested at such a stage (12).

Two clones of brome grass, OM-1 and Cl 23, flowered only on short photoperiod in experiment I and only on long photoperiod in experiment II. Since, however, they flowered 45 and 30 days earlier, respectively, on long photoperiod than on short, it is reasonable to assume that the longer photoperiod is more favorable for the completion of reproductive activity. The fact that both OM-2 and Cl 84 flowered on 18-hour photoperiod in 1947 and failed to flower on a 17-hour photoperiod in 1946 could be interpreted to mean that the lower critical limit for flowering of these clones lies between 17 and 18 hours. However, it is perhaps more reasonable to believe that the large pieces of clonal material used in 1947 were more representative of the state of the entire plant at the time of bringing it in than were the single pieces of rhizome used in 1946. Growing-points of the segments used in 1946 may not have been "ripe-to-flower" because of age, position on the plant, or other factor.

Vegetative behavior under the conditions of these experiments was consistent

with that reported by others for brome grass (1, 3, 14) and for other species of grasses (1, 8, 11, 15). Plants on short photoperiods tillered more abundantly than those on long photoperiods and showed little stem elongation. Shoots produced on short photoperiod were generally decumbent or semi-decumbent, resulting in a rosette type of plant. On 18-hour photoperiod marked elongation of sterile shoots occurred in the August plants of experiment II, so that at harvest the plants closely resembled the types called "sterilclinous" by WALDRON (13). This also occurred in the 18-hour December plants in two or three instances (fig. 6) in which sterile shoots continued growth after maturation of the panicles on other culms and finally overtopped the flowering stems.

Growth and flowering responses of brome grass in these experiments showed no apparent correlation with place of origin of the clones. Differences of a few days in time of flowering cannot be held significant when the approximate nature of the data is considered. The thirteen clones behaved in a somewhat similar manner physiologically under the experimental conditions, in spite of their diversity in origin and habit, with the possible exception of the Manchurian clone, Cl 9. This plant was consistently the first to flower on long photoperiods and bloomed also on 15- and 13-hour light periods. It has flowered as well without preconditioning by chilling. Vegetatively it was the tallest of the clones and produced the greatest weight of both tops and roots.

It is apparent that a combination of conditions is necessary to insure most vigorous growth, flowering, and production of seed of brome grass in the greenhouse in winter. In these experiments these ends were accomplished most successfully when the plants were brought in from the field after a period of chilling and were allowed to grow in the pots for only 10 days before being subjected to a relatively high intensity of supplementary light and long photoperiods. Fairly large pieces of transplant material were more successful than single rhizomes.

Summary

1. Flowering and growth responses of thirteen diverse clones of smooth brome grass were studied in greenhouse experiments, supplemented by observations made on plants established in the field, conducted during the winter and spring of 1946 and 1947.

2. In early 1946, clonal divisions, consisting of single sections of rhizomes, were grown on Chicago natural daylength and light intensity and on 17-, 15-, and 13-hour photoperiods under fluorescent lamps providing relatively high light intensity and under incandescent-filament lamps providing relatively low light intensity. Flowering first occurred approximately 1 month after bringing the plants in from the field in early January. Earliest and most prolific flowering and most vigorous vegetative growth occurred on 17-hour photoperiod under fluorescent light. The number of elongated tillers was directly, and of unelongated tillers inversely, correlated with length of photoperiod. Rhizome production was not obviously affected by photoperiod but was strongly affected by light intensity.

3. Plants were brought in from the field on August 31, 1946, and again on December 27 after exposure to low temperatures. Clonal divisions of both groups of plants, consisting of pieces of entire plants, were grown on 18- and 13-hour photoperiods under fluorescent lamps, beginning January 7, 1947, and on natural daylength. Flowering of the 18-hour De-

cember plants occurred in approximately 3–4 weeks after differential photoperiodic treatments were begun. One December plant on 13-hour photoperiod flowered. No December plants on natural daylength had flowered by June 1. None of the August plants flowered under any treatment, but all on long photoperiod showed marked tiller elongation. Increase in number of tillers was greatest for the August plants on natural daylength, least for those on 18-hour photoperiod. Of the December plants, the series on natural daylength produced most new tillers, plants on 13-hour photoperiod least. Dry weights of tops of both August and December plants were greatest in the 18-hour series, of roots in the 13-hour series. Height in both August and December plants was greatest in the 18-hour series, least in the series on natural daylength. Fully viable seed was produced on the 18-hour December plants under the relatively poor growing conditions of a Chicago winter in the greenhouse.

4. Floral primordia were present on plants growing in the field at Chicago in early April. A 13-hour photoperiod inhibited their subsequent normal development and elongation.

5. The vegetative and flowering responses showed no apparent correlation with place of origin of the clones.

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REACTION OF CERTAIN PLANT GROWTH-REGULATORS WITH ION EXCHANGERS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 589

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Introduction

Several investigators have reported that 2,4-dichlorophenoxyacetic acid (2,4-D) and certain other plant growth-regulators are readily leached from soils (2, 3, 7). NUTMAN *et al.* (7) found that leaching equivalent to 1.4 inches of water resulted in a loss of 2,4-D from the soil but that far greater volumes of water did not completely remove it. This would indicate that some 2,4-D may be adsorbed by the soil. HANKS (3) studied rates of leaching of 2,4-D and its calcium salt from six types of soil and found that the two compounds were leached from any one soil at about equal rates, as shown by comparative toxicities of the leachates, but were differentially leached from the various soils. It was recently shown by LUCAS and HAMNER (5) that 2,4-D is inactivated by adsorption on charcoal. Bean plants sprayed with a 0.1% solution of sodium 2,4-dichlorophenoxyacetate, which had been mixed and shaken with 1% activated charcoal, showed little injury.

The degree of adsorption of several growth-regulators by certain ion-exchange materials and also the readiness with which the compounds are eluted after having been adsorbed are reported here. Growth of plants in exchanger materials containing an adsorbed growth-regulator was also observed. Such studies may help to explain why herbicidal growth-regulators vary in toxicity in different soils and why the rates of leaching of the compounds from different soils may vary. A spectrophotometric method developed by BANDURSKI (1) for determining growth-regulators in solution made possible quantitative studies of adsorption and leaching of such compounds which could heretofore be done only with difficulty when using bioassays (6, 8).

Material and methods

The cation exchangers used were a resin exchanger (Amberlite IR-100), a carbonaceous exchanger (Zeo-Karb H), a synthetic sodium alumino-silicate (Decalso), and a processed glauconite (Zeo-Dur). The anion exchangers were amine resins (Amberlite IR-4B and De-Acidite). The Amberlites were obtained from the Resinous Products and Chemical Company, and other exchangers from the Permutit Company. Hereafter the word "Amberlite" is omitted when this type of exchanger is mentioned.

All cation exchangers were screened so that the size of the particles was smaller than 20 mesh but larger than 40 mesh. IR-100 and Zeo-Karb H were stirred with 5% hydrochloric acid, which was changed several times, for a period of 48 hours. Decalso, Zeo-Dur, and IR-100 were treated with several changes of 4% sodium chloride or calcium chloride solution over a period of 24 hours. The materials were then washed thoroughly with distilled water until free of hydrochloric acid or the chloride salts. They were dried in an oven at 40° C. and then placed in tightly stoppered bottles.

The anion exchangers were prepared by stirring with several changes of 5%

sodium carbonate solution over a 24-hour period. The materials were then dried for about 5 hours at 40° C. and placed in tightly stoppered bottles.

Moisture contents of the prepared materials were determined by heating ½-gm. samples in an oven at 100° C. (table 1) to make possible the calculation of the capacities of the various exchangers on a comparative basis.

Six plant growth-regulators were used for experimentation: 2,4-dichlorophenoxyacetic acid (2,4-D), ammonium 2,4-dichlorophenoxyacetate ($NH_4$2,4-D), cupric 2,4-dichlorophenoxyacetate [Cu(2,4-D)$_2$], calcium 2,4-dichlorophenoxyacetate [Ca(2,4-D)$_2$], 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), and isopropyl N-phenylcarbamate (IPPC). The 2,4-D was purified by running it through several salt-acid cycles. Cu(2,4-D)$_2$ was prepared by causing an excess of an aqueous solution of $NH_4$2,4-D to react with a solution of cupric chloride, and then washing the precipitate of Cu(2,4-D)$_2$ free of ammonium chloride. Ca(2,4-D)$_2$ was made by adding calcium chloride to an aqueous solution of $NH_4$2,4-D. The 2,4,5-T and the IPPC used were from commercial sources and were assumed to be relatively pure. The purity of 2,4-D and its salts was confirmed by observation of the molecular extinction values of the complexes as established with the spectrophotometer.

TABLE 1

MOISTURE CONTENT OF EXCHANGE MATERIALS

Exchange material	Cycle	% moisture
Amberlite IR-100 H	H^+	12.4
Amberlite IR-100 Na	Na^+	5.6
Amberlite IR-100 Ca	Ca^{++}	6.0
Zeo-Karb H	H^+	5.8
Decalso	Na^+	7.6
Zeo-Dur	Na^+	7.6
Amberlite IR-4B	Anion	10.6
De-Acidite	Anion	36.6

The spectrophotometric method developed by BANDURSKI (1) was employed to measure the compounds in solution. A quartz spectrophotometer, Beckman model DU, using a hydrogen discharge tube as the light source and silica sample cells, was employed. The absorption maximum of 2,4,5-T in water was located at 2,880 A and that of IPPC at 2,690 A. The absorption maximum of 2,835 A was used for 2,4-D in water (1).

PROCEDURE FOR STATIC TRIALS

Static trials were divided into two types: (*a*) those in which it was determined how much of a compound an exchanger material removed from a solution and (*b*) those in which elution of regulators from exchangers was studied.

In static trials 0.5-gm. samples of the exchanger were placed in 250-ml. Erlenmeyer flasks. Fifty milliliters of the appropriate solution were pipetted into the flask, which was then stoppered. The flask was allowed to stand for 48 hours with frequent shaking. At this time the concentration of the growth-regulator in the supernatant liquid was determined, and the amount of compound adsorbed or eluted was calculated. Each test was run in duplicate or triplicate.

In elution studies the compounds were usually added to the exchangers by dissolving the growth-regulators in 95% ethyl alcohol and shaking with the exchangers for about 30 minutes. The alcohol was then evaporated by placing the container in a circulating oven at about 70° C. The growth-regulators were also added to exchanger materials by shaking the exchangers with aqueous solutions of the compounds. The amount of regulator adsorbed by the exchanger was calculated by measuring the decrease in con-

centration of the compound in the supernatant liquid.

PROCEDURE FOR LEACHING EXPERIMENTS

Pyrex-glass tubes about 40 mm. in diameter and 25 cm. in height were used for studies of leaching (fig. 1). The tubes were tapered at the lower end so that the bottom 5 cm. were about $\frac{1}{4}$ inch in diameter. The exchanger bed in each tube, about 10 cm. in height, was supported by a plug of glass wool at the point of constriction in the tube. A piece of rubber

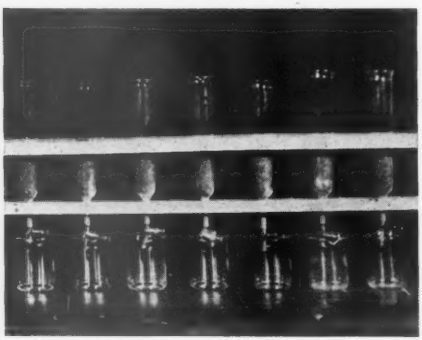

FIG. 1.—Equipment for leaching experiments

tubing about $1\frac{1}{2}$ inches long and with a screw-type clamp was fitted over the lower end of the leaching tube. A reservoir of liquid could thus be maintained above the exchanger material.

After the exchanger material was placed in the tube, distilled water was slowly forced into the lower end of the tube until the level of water reached the top of the exchanger bed; the screw clamp was then closed. This backwashing was necessary in order to prevent presence of air pockets in the bed. After allowing to stand for 15 minutes, 55 ml. (a 2-inch column) of distilled water was added above the bed. The screw clamp was then opened so that a slow drip was obtained which allowed 55 ml. of water to leach through in about 45 minutes, after which the clamp was closed. During leaching the tubes were frequently rotated so that the flow rate would be uniform throughout the bed. All tests were run in triplicate.

Experimentation

The objective of one experiment was to determine the relative adsorptive capacities of six exchanger materials for six growth-regulators. Aqueous solutions of 0.000226 normality of 2,4-D (50 p.p.m.), $NH_4$2,4-D, Cu(2,4-D)$_2$, Ca(2,4-D)$_2$, 2,4,5-T, and IPPC were prepared. IPPC was considered to have a valence of one. Fifty milliliters of these solutions were added to 0.5-gm. samples of air-dry IR-100 H, Zeo-Karb H, Decalso (sodium cycle), Zeo-Dur (sodium cycle), or De-Acidite. For IR-4B, 0.439-gm. samples were used. The amount of regulators adsorbed was determined, using procedures described for static trials (table 2).

Data of table 2 indicate that IR-100 H and Zeo-Karb H adsorbed much of the growth-regulators, while the Decalso and Zeo-Dur adsorbed little or none. The anion exchangers, especially IR-4B, adsorbed much of the growth-regulators. The IR-100 H adsorbed almost equal micro-equivalents of 2,4-D and its salts. De-Acidite and IR-4B, however, adsorbed less of Cu(2,4-D)$_2$ than of 2,4-D, Ca(2,4-D)$_2$, or $NH_4$2,4-D. IR-100 H adsorbed less of 2,4,5-T than of 2,4-D or its salts but adsorbed much more IPPC. With the anion exchangers the situation was reversed; there was a relatively large amount of 2,4,5-T adsorbed and a small amount of IPPC.

The results of this experiment showed that growth-regulators were strongly adsorbed by cation exchangers in the hydrogen cycle but that little or no adsorp-

TABLE 2

ADSORPTION OF SIX PLANT GROWTH-REGULATORS BY SIX EXCHANGER MATERIALS
EACH FIGURE IS AN AVERAGE OF THREE REPLICATES

Growth-regulator	Micro-equivalents adsorbed	Mg. adsorbed per ½-gm. oven-dry exchanger	% adsorbed	Micro-equivalents adsorbed	Mg. adsorbed per ½ gm. oven-dry exchanger	% adsorbed	Micro-equivalents adsorbed	Mg. adsorbed per ½ gm. oven-dry exchanger	% adsorbed
	IR-100 H			Zeo-Karb H			Decalso		
2,4-D	5.78	1.46	51.1	11.0	2.58	97.3	0	0	0
$NH_4$2,4-D	5.97	1.62	52.8	11.0	2.78	97.3	0	0	0
Cu(2,4-D)$_2$	5.72	1.64	50.6	11.0	2.93	97.3	0	0	0
Ca(2,4-D)$_2$	6.13	1.68	54.2	11.1	2.82	98.1	0.27	0.70	2.39
2,4,5-T	4.77	1.39	42.2	11.0	2.98	97.3	0	0	0
IPPC	8.70	1.78	76.9	11.2	2.13	99.0	0.45	0.87	3.98
	Zeo-Dur			De-Acidite			IR-4B		
2,4-D	0	0	0	5.23	1.83	46.2	9.05	2.53	80.0
$NH_4$2,4-D	0.16	0.041	1.41	5.08	1.91	44.9	8.45	2.55	74.7
Cu(2,4-D)$_2$	.01	.003	0.09	3.42	1.36	30.2	7.70	2.46	68.1
Ca(2,4-D)$_2$	.18	.047	1.59	5.49	2.08	48.5	8.60	2.62	76.0
2,4,5-T	.73	.201	6.45	6.17	2.49	54.6	9.21	2.99	81.4
IPPC	0.43	0.083	3.80	1.03	0.29	9.1	5.47	1.24	48.4

tion occurred with those in the sodium cycle. Another experiment was performed to determine how much 2,4-D or IPPC would be adsorbed by IR-100 in the hydrogen, sodium, or calcium form. It was shown that much IPPC is adsorbed by all exchangers but that only the hydrogen form adsorbed much 2,4-D (table 3). Each test was made in duplicate.

Another experiment was run to determine whether a hydrogen exchanger adsorbs 2,4-D mainly by virtue of its properties as an acid. Fifty-milliliter aliquots containing 40 p.p.m. of 2,4-D in the presence of varying amounts of hydrochloric acid were added to 0.5-gm. samples of IR-100 H. The solutions were adjusted to five different degrees of acidity. A series of blanks was run with acidulated water adjusted to the same pH as the solutions of 2,4-D. Much less 2,4-D was adsorbed when the pH of the solution was 3.3 than when it was 2.5 or lower (table 4).

In another experiment in which varying concentrations of 2,4-D were added to 0.5-gm. samples of IR-100 H or IR-4B, the amount of 2,4-D adsorbed by the ion exchangers was approximately in direct proportion to the initial concentration of 2,4-D (table 5). The percentage of 2,4-D adsorbed was about the same at all concentrations used.

TABLE 3

ADSORPTION OF 2,4-D AND IPPC BY IR-100 H, IR-100 NA, AND IR-100 CA FROM 0.000226 *N* AQUEOUS SOLUTIONS OF THE COMPOUNDS

GROWTH-REGULATOR	% OF REGULATOR ADSORBED		
	IR-100 H	IR-100 Na	IR-100 Ca
2,4-D	57.9	0.44	3.2
IPPC	81.4	74.50	70.0

It was attempted to elute 2,4-D from IR-100 H with 0.75 *N* solutions of HCl, NaCl, $CaCl_2$, or $AlCl_3 \cdot 6H_2O$. The 2,4-D was put on the exchanger with alcohol so that there was about 1.5 mg. of 2,4-D per 0.5 gm. of air-dry resin. The solutions were also added to exchangers which contained no 2,4-D. The latter were used as blanks. Each test was run in duplicate. It was found that the presence of the salts decreased the quantity of 2,4-D eluted as compared with that eluted by distilled water (table 6). Hydrochloric acid had little effect upon the amount of elution. Since it was thought that the method of application of 2,4-D to the exchanger with alcohol may have affected its exchange properties, some 2,4-D was applied to the exchanger in aqueous solution. When the exchanger containing 2,4-D was shaken with a 0.75 *N* solution of sodium chloride, results similar to those shown in table 6 were obtained.

TABLE 4

ADSORPTION OF 2,4-D BY IR-100 H IN THE PRESENCE OF HYDROCHLORIC ACID

Initial pH	% of 2,4-D adsorbed
3.3	52.4
2.5	89.4
2.0	89.4
1.7	93.2
1.4	89.4

Elution of 2,4-D from ion exchangers by use of anions of varying valences was attempted. Solutions of 0.685 normality of NaCl, Na_2SO_4, and $Na_3PO_4 \cdot 12H_2O$ were prepared and were added to IR-100 H or Zeo-Karb H containing, respectively, 1.5 or 2.5 mg. of 2,4-D per 0.5 gm. of air-dry exchanger. Each test was made in duplicate, and blanks were run on exchangers containing no 2,4-D. Less 2,4-D was eluted from IR-100 H by the salts than by distilled water, and, the higher the valence of the anion, the smaller was the amount of 2,4-D eluted (table 7). No 2,4-D was eluted from Zeo-Karb H.

It was shown that IPPC could be eluted from IR-100 H by 0.685 *N* sodium chloride. The growth-regulator was applied to IR-100 H or to Zeo-Karb H in alcohol so that each contained, respectively, 1.5 or 2.5 mg. of IPPC per 0.5 gm. of air-dry exchanger. The sodium chloride solution eluted 33.3% of the growth-regulator fron IR-100 H, while water eluted none. No IPPC was eluted from Zeo-Karb H.

TABLE 5

ADSORPTION OF 2,4-D BY IR-100 H AND IR-4B FROM SOLUTIONS OF VARYING CONCENTRATION

Exchanger	Initial conc. of 2,4-D (p.p.m.)	Mg. 2,4-D adsorbed by ½-gm. oven-dry exchanger	% adsorbed
IR-100 H	20	0.63	63.0
	50	1.46	58.4
	100	.....	.....
	200	6.85	68.5
IR-4B	20	0.89	89.0
	50	2.06	82.4
	100	4.09	81.8
	200	7.97	79.7

The following experiment shows that 2,4-D is readily eluted from the anion exchangers, IR-4B and De-Acidite, by a suitable concentration of hydrochloric acid. IR-4B and De-Acidite were prepared which contained, respectively, 1.39 and 1.16 mg. of 2,4-D per 0.5 gm. of air-dry exchanger. To 0.5-gm. samples of the resins were added 0.126 or 0.00126 *N* hydrochloric acid. The stronger concentration of acid readily replaced the adsorbed 2,4-D (table 8).

LEACHING EXPERIMENTS

Static trials showed that 2,4-D was strongly adsorbed by IR-100 H and Zeo-

Karb H and that the compound was not readily eluted from the exchangers by water or salt solutions. It was further shown that Decalso and Zeo-Dur adsorbed little or no 2,4-D. Since many soils may have a low adsorptive capacity for 2,4-D, an experiment was set up to determine how readily 2,4-D is leached from Decalso and Zeo-Dur.

The sodium forms of the exchangers were used. The 2,4-D was applied to the exchangers at two rates: 19.4 and about 64 mg. per pound on a dry-weight basis. Beds of Decalso 10 cm. deep contained 3 or 10 mg. of 2,4-D while beds of Zeo-Dur contained 5.17 or 17.0 mg. Some tubes used as blanks contained beds with no 2,4-D. Each bed received three successive leachings of 55 ml. of distilled water. The second and third leachings of the beds of Decalso were made 2 and 4 hours after the first; with Zeo-Dur, in about 24 and 48 hours after the first.

Much 2,4-D was removed by the first leaching, a smaller amount by the second leaching, while practically none was found in the third leachate (table 9). In

TABLE 6

ELUTION OF 2,4-D FROM 0.5-GM. SAMPLES OF IR-100 H BY WATER AND BY 0.75 *N* SOLUTIONS CONTAINING A MONO-, DI-, OR TRIVALENT CATION

Solution	Mg. of 2,4-D eluted	% of 2,4-D eluted
Water	0.685	45.7
HCl	.720	48.0
NaCl	.335	22.3
$CaCl_2$	.370	24.7
$AlCl_3 \cdot 6H_2O$	0.436	29.1

no case was more than 90% of the 2,4-D eluted by the 6 inches of water applied, and usually the percentage was much less.

An experiment was run to compare the rate of leaching of six growth-regulators from Decalso and Zeo-Dur. IPPC, 2,4-D, the ammonium, calcium, and cupric salts of 2,4-D, and 2,4,5-T were the compounds used. All compounds except IPPC were applied to Decalso at the rate of 600 micro-equivalents per pound and

TABLE 7

ELUTION OF 2,4-D FROM 0.5-GM. SAMPLES OF IR-100 H BY WATER AND BY 0.685 *N* SOLUTIONS CONTAINING A MONO-, DI-, OR TRIVALENT ANION

Solution	Mg. of 2,4-D eluted	% of 2,4-D eluted
Water	0.976	65.1
NaCl	.420	28.0
Na_2SO_4	0.385	25.7
$Na_3PO_4 \cdot 12H_2O$	0	0

to Zeo-Dur at the rate of 342 micro-equivalents per pound. IPPC was applied to Decalso and Zeo-Dur at rates of 660 and 376 micro-equivalents per pound, respectively. A bed of each type of exchanger contained 102.3 micro-equivalents of IPPC or 93.0 micro-equivalents of one of the other compounds.

Each bed was leached with 2 inches of water. Usually more than one-half of each regulator was leached from each exchanger (table 10). All compounds were leached from the exchanger materials in about the same amounts. An exception was IPPC on Zeo-Dur, which was leached to a much lower degree.

PLANT GROWTH IN ION EXCHANGERS

Experiments were conducted to determine the toxicity to plants of $NH_4$2,4-D adsorbed on exchange materials mixed in soil in which they were grown. In one experiment the emergence of seedlings from soils containing $NH_4$2,4-D adsorbed on a strong adsorbent (Zeo-Karb H) or on a weak adsorbent (quartz sand) was studied. One hundred

milliliters of 2.0, 0.2, 0.02, or 0.002% aqueous solutions of $NH_4$2,4-D were added to 60 gm. of air-dry Zeo-Karb H or to quartz sand in 250-ml. beakers. The mixtures were stirred occasionally over a 2-hour period and then were dried in an oven at 50° C. Each sample was then thoroughly mixed with about 1.87 lb. of a potting soil (three parts soil to one part fine gravel). Thus mixtures of soil and Zeo-Karb H or sand were obtained which contained about 0, 1, 10, 100, or 1,000 mg. of $NH_4$2,4-D per pound of soil when calculated on an air-dry basis. The soils were placed in 4-inch unglazed clay pots, which were set on a greenhouse bench. Fifteen seeds of barley and white mustard were planted in each pot. Each treatment consisted of three replicate pots.

TABLE 8

ELUTION OF 2,4-D FROM IR-4B AND DE-ACIDITE BY HYDROCHLORIC ACID

SOLUTION	IR-4B		DE-ACIDITE	
	Mg. of 2,4-D eluted	% of 2,4-D eluted	Mg. of 2,4-D eluted	% of 2,4-D eluted
Water	0.000	0	0.03	0.02
0.00126 *N* HCl	0	0	0.03	0.02
0.126 *N* HCl	0.578	41.6	1.10	94.83

Seven days after planting, counts were made of seedlings appearing above the soil surface (table 11). The data indicate that there was only a limited or no toxicity to the germinating seedlings from the low concentrations of $NH_4$2,4-D adsorbed on Zeo-Karb H but that toxicity was strong at the high concentration. Perhaps much of the $NH_4$2,4-D was strongly adsorbed by the ion exchanger, and the growth-regulator in an adsorbed condition could not affect plant growth. White mustard germinated normally in soil–Zeo-Karb H mixtures containing $NH_4$2,4-D at a rate of 10 mg. per pound of soil, but, when sand only was used as the carrier for the growth-regulator, the lowest concentration (1 mg. / lb.) prevented germination. Barley germinated well in soil–Zeo-Karb H mixtures when $NH_4$2,4-D was present at the concentration of 100 mg. per pound, but with sand as the carrier 10 mg. of $NH_4$2,4-D per pound of soil reduced the number of emerging barley seedlings.

In another experiment the growth of plants in exchangers containing high con-

TABLE 9

LEACHING OF 2,4-D FROM DECALSO AND ZEO-DUR BY 2-INCH COLUMNS OF WATER. FIGURES ARE AVERAGES OF THREE REPLICATES

EXCHANGER	MG. OF 2,4-D/LB.	LEACHING					
		First		Second		Third	
		Mg. of 2,4-D eluted	% of 2,4-D eluted	Mg. of 2,4-D eluted	% of 2,4-D eluted	Mg. of 2,4-D eluted	% of 2,4-D eluted
Decalso	19.4	0.960	32.00	0.528	17.60	0.011	0.37
	64.7	4.070	40.70	0.950	9.50	.055	.55
Zeo-Dur	19.4	2.900	56.09	1.600	30.95	.025	.48
	63.8	7.900	46.47	3.000	17.65	0.055	0.32

centrations of adsorbed $NH_4$2,4-D was observed. Aqueous solutions of $NH_4$2,4-D were added to Zeo-Karb H and IR-100 H, and the mixtures were dried at 70° C. The dried materials were then thoroughly mixed with two parts by weight of fine gravel. The gravel–IR-100 H mixture contained 0, 3, 30, or 300 mg. of $NH_4$2,4-D per pound, and the gravel–Zeo-Karb H mixture 0, 1, 10, 100, or 1,000 mg. of $NH_4$2,4-D per pound on a dry-weight basis. The mixtures were placed in 4-inch unglazed clay pots, and fifteen seeds of barley and white mustard were planted in each pot.

TABLE 10

LEACHING OF SIX GROWTH-REGULATORS FROM DECALSO AND ZEO-DUR BY A 2-INCH COLUMN OF WATER. FIGURES ARE AVERAGES OF THREE REPLICATES

COMPOUND	INITIAL CONC. PER BED (MICRO-EQ.)	DECALSO			ZEO-DUR		
		Micro-eq. eluted	Mg. eluted	% eluted	Micro-eq. eluted	Mg. eluted	% eluted
2,4-D	93	54.8	12.11	58.9	62.3	13.77	67.0
$NH_4$2,4-D	93	52.5	12.50	56.5	60.6	14.42	65.2
$Ca(2,4\text{-}D)_2$	93	49.8	11.95	53.5	47.8	11.47	51.4
$Cu(2,4\text{-}D)_2$	93	56.2	14.15	60.4	54.2	13.64	58.3
2,4,5-T	93	51.7	13.21	55.6	45.2	11.55	48.6
IPPC	102.3	60.0	10.74	58.7	29.2	5.23	28.5

TABLE 11

AVERAGE NUMBER OF WHITE MUSTARD AND BARLEY SEEDLINGS PER POT EMERGED FROM SOILS CONTAINING VARIOUS AMOUNTS OF $NH_4$2,4-D APPLIED TO SOIL ON ZEO-KARB H OR QUARTZ SAND. THREE REPLICATES

PLANT	CARRIER	CONCENTRATION OF $NH_4$2,4-D (MG./LB.)				
		0	1	10	100	1,000
White mustard	Zeo-Karb H	9.0	9.7	10.3	1.0	0.0
	Sand	10.7	0.0	0.0	0.0	0.0
Barley	Zeo-Karb H	11.0	6.7	8.3	9.7	0.0
	Sand	9.0	7.3	3.3	0.3	0.0

The plants were harvested 19 days after planting (fig. 2, table 12). White mustard plants had developed two true leaves in control gravel–Zeo-Karb H mixtures. Mustard plants also developed in mixtures containing 1 or 10 mg. of $NH_4$2,4-D per pound. The barley in control mixtures was in the second leaf stage and had an average foliage height of 6 inches. It had developed well in all gravel–Zeo-Karb H mixtures except those containing 1,000 mg. of growth-regulator per pound of mixture. The stubby roots of the barley growing in the latter mixture were usually less than 1 cm. in length (fig. 3).

White mustard failed to germinate in the gravel–IR-100 H mixture. The average fresh weights of tops of barley on a

one-pot basis for control gravel–IR-100 H mixture and for mixtures containing 3, 30, or 300 mg. of $NH_4$2,4-D per pound were 1.59, 1.55, 0.27, and 0.09 gm., respectively. The root systems of barley in the mixtures treated with $NH_4$2,4-D at a rate of 30 mg. per pound were small, and many of the roots were ribbon-like (fig. 3). The relative growth of barley in the gravel–Zeo-Karb H and gravel–IR-100 H mixtures indicates that $NH_4$2,4-D is less toxic when adsorbed on Zeo-Karb H than on IR-100 H. This is probably associated with the greater adsorptive capacity of Zeo-Karb H for $NH_4$2,4-D than is possessed by IR-100 H.

Discussion

Cation exchangers in the hydrogen cycle strongly adsorbed all the growth-regulators studied. For example, IR-100 H adsorbed 2,4-D from a 0.02% solution at a rate of more than 6 gm. per pound of exchanger. It seems probable that when 2,4-D or its salts are added to soils in chemically equivalent quantities, ap-

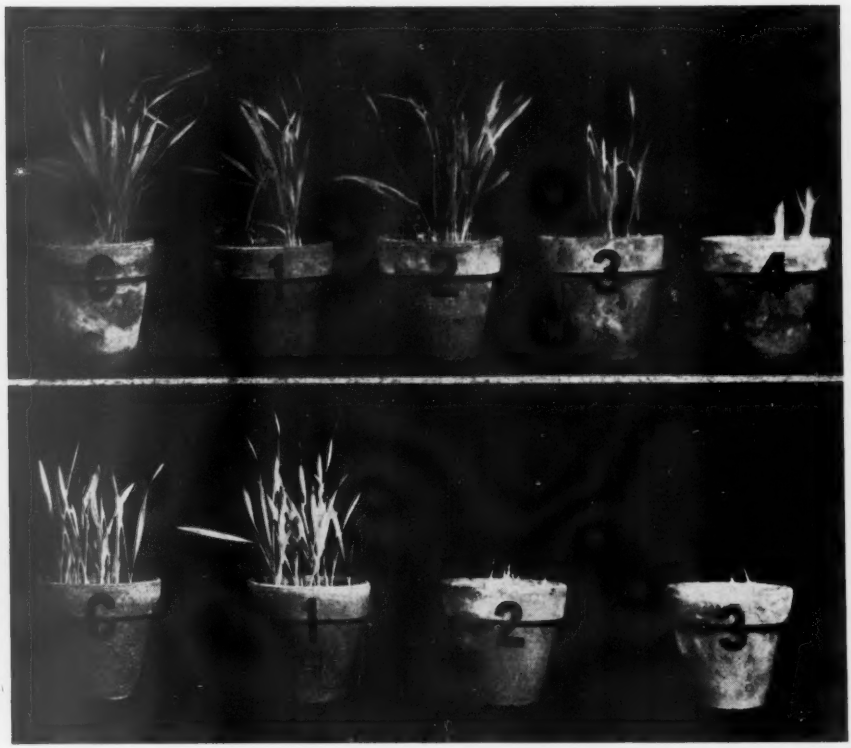

FIG. 2.—White mustard and barley plants grown in mixtures of (*upper*) Zeo-Karb H and gravel and (*lower*) Amberlite IR-100 H and gravel. *Left to right:* Zeo-Karb H mixtures contained 0, 1, 10, 100, and 1,000 mg. of $NH_4$2,4-D per pound; Amberlite mixtures contained 0, 3, 30, and 300 mg. of $NH_4$2,4-D per pound. White mustard seedlings grew normally in Zeo-Karb H mixtures containing 10 mg. of $NH_4$2,4-D per pound, and barley made much growth in Zeo-Karb H mixtures containing 100 mg. of growth-regulator per pound. Photographed 19 days after planting.

proximately equal amounts of the anion of each would be adsorbed by the soil. Such equivalent adsorption might not hold true in soils containing anion- or acid-exchanging materials in large amounts, as less $Cu(2,4\text{-}D)_2$ was adsorbed by the anion exchangers than was 2,4-D or its ammonium or calcium salts.

The high adsorptive capacities of certain ion exchangers for growth-regulators, combined with the fact that salt solutions failed to elute all the 2,4-D from cation exchangers, may be of great significance when growth-regulators are applied as herbicides. When the adsorptive capacity of a soil is high, there may be less loss of the compound from the

TABLE 12

AVERAGE FRESH WEIGHTS (GM.) PER POT OF TOPS OF BARLEY AND WHITE MUSTARD GROWN IN GRAVEL–ZEO-KARB H MIXTURES CONTAINING VARIOUS CONCENTRATIONS OF $NH_4$2,4-D. BASED ON THREE REPLICATES

PLANT	CONC. OF $NH_4$2,4-D (MG./LB.)				
	0	1	10	100	1,000
White mustard	0.63	0.68	0.23	0.02	0.00
Barley	1.96	2.05	2.28	1.36	0.22

FIG. 3.—Barley seedlings grown in mixtures of (*upper*) Zeo-Karb H and gravel containing 1,000 mg. of $NH_4$2,4-D per pound and of (*lower*) Amberlite IR-100 H and gravel containing 30 mg. of growth-regulator per pound. Roots in Zeo-Karb H mixtures were short and stubby. Many roots in Amberlite mixtures were flattened and had many small gall-like protuberances.

soil by leaching. But, when adsorbed, such material may be not particularly toxic to plants grown in the soil. For example, it was shown that much $NH_4$2,4-D adsorbed on Zeo-Karb H was nontoxic to plants growing in mixtures of the ion exchanger and gravel. Barley plants grew well in gravel–Zeo-Karb H mixtures containing 100 mg. of $NH_4$2,4-D per pound of mixture. The growth-regulator was evidently strongly adsorbed, since a surface application of $NH_4$2,4-D of 100 mg. per 12.5 sq. in. (approximate surface area of a 4-inch clay pot) corresponds to a rate of about 110 pounds per acre, which is far in excess of any field application. It is possible that the compound did not come into contact with the roots growing around the adsorbent or perhaps could not diffuse into or enter the plants.

The varying toxicity of a growth-regulator in different soils may be associated with the differences in adsorptive capacities of the soils for the various regulators. Since adsorptive capacities of soils may affect toxicity of a growth-regulator in them, it is evident that any modification of such capacities through agricultural practices might be very important. Because soils are very complex systems, it is difficult to say which factors are most concerned with the degree of toxicity and with the fate of a growth-regulator in a soil. In a soil of high adsorptive capacity, one would expect greater retention (less leaching) of the compound, but the amount present in such a condition as to affect plant growth might not necessarily be correspondingly high. Much of the adsorbed compound might be nontoxic to plants. The rate of decomposition of the compounds in soil is of great importance. It would be of interest to know whether adsorbed compounds are subject to the same decomposition processes and rates as the nonadsorbed. A desirable carrier for 2,4-D for certain uses might be one with a high adsorptive capacity which would slowly release the compound over a long period of time.

The effectiveness of any herbicidal practice involves many details regarding herbicides, crops, and soils. The fate of a growth-regulator in soil is probably just as much influenced by agricultural practices as is the amount and availability of nutrients and the activity of organic constituents. Thus many interlocking factors may determine the toxicity and duration of a growth-regulator in a soil. By the use of a suitable system of soil management, the toxicity and persistence of a compound in the soil may be modified. The indication is that acidic soils might have higher adsorptive capacities for 2,4-D or its salts than alkaline ones. Cation exchangers in the sodium or calcium form adsorbed little or no 2,4-D. The respiration of plant roots and the microflora in a soil may result in changes in the soil pH during the season which might affect adsorption of growth-regulators. Soil reaction can be changed by application of ammonium sulfate, lime, or other compounds. It is possible that various compounds may be found in the soil which would elute growth-regulators from the soil adsorbents and would also change the degree of solubility of the herbicides in the soil solution. The persistence of toxicity of growth-regulators in soil may be partially controlled by addition of organic matter or by liming. KRIES (4) has shown that addition of lime to soil decreased the rate of disappearance of the toxic effects of 2,4-D, while the addition of organic matter counteracted the effect of the lime.

A chemical or physical explanation of the adsorption of 2,4-D and its salts by

the cation exchangers is difficult with the data available. 2,4-D is probably eluted from anion exchangers in a typical acid or anion exchange reaction, since 2,4-D is readily eluted by hydrochloric acid:

$$RNH_3(2,4\text{-D}) + HCl \leftrightharpoons RNH_3\,Cl + H2,4\text{-D}\,.$$

IPPC differs from 2,4-D in that much more IPPC is adsorbed by cation exchangers, and much less by anion exchangers, and it may be eluted from IR-100 H by sodium chloride. It is also adsorbed by the sodium and calcium forms of IR-100. IPPC may react with IR-100 H by physical adsorption or by salt formation with the basic amino group:

$$RH + \phi NHCOOR \leftrightharpoons RN\phi H_2COOR\,.$$

The experiments in leaching using beds of Decalso and Zeo-Dur showed that the growth-regulators are readily leached from exchange materials having little or no adsorptive capacity. The various compounds were usually removed in about the same amounts. Even the most soluble compound used, $NH_4$2,4-D, was not removed in greater quantities than 2,4-D, which is of much lower solubility. Leaching experiments in which smaller quantities of water were used might result in differential rates of leaching of the various compounds based on their solubilities. It seems probable, however, that in the soil 2,4-D or a relatively insoluble 2,4-D salt might be converted to soluble forms by reaction with ammonium, sodium, or other ions present in the soil solution.

Experiments in which exchanger beds were successively leached with 2-inch columns of water showed that 4 inches of water removed practically all the 2,4-D that could be leached from the beds. In the case of Decalso beds this amounted to only about one-half of the total amount of the growth-regulator initially in the bed. It may be that the Decalso adsorbed some 2,4-D because of the method of application of the compound to the exchanger. The important point, however, is the rapidity with which 2,4-D was removed from the beds by leaching.

Summary

1. Studies were made on the adsorption by and elution from several ion exchangers of six plant growth-regulators. Cation exchangers in the hydrogen cycle adsorbed much 2,4-dichlorophenoxyacetic acid (2,4-D) and its ammonium, calcium, and cupric salts, and in about equal amounts. Anion exchangers adsorbed much 2,4-D and its salts, but not always in equal amounts. Little or no 2,4-D or its salts was adsorbed by cation exchangers in the sodium or calcium form. 2,4,5-Trichlorophenoxyacetic acid was adsorbed in amounts similar to those of 2,4-D except that less of the former was adsorbed by the cation exchangers.

2. Isopropyl N-phenylcarbamate was more strongly adsorbed than 2,4-D by cation exchangers in the hydrogen, sodium, or calcium form. Much less was adsorbed by the acid exchangers.

3. Much less 2,4-D was adsorbed by Amberlite IR-100 H when the pH of the solution was 3.3 than when it was 2.5 or lower. The amount of 2,4-D adsorbed by ion exchangers from solutions varying in concentration from 20 to 200 p.p.m. was in about direct proportion to the concentration of 2,4-D in the initial solution.

4. Solutions of NaCl, $CaCl_2$, or $AlCl_3 \cdot 6H_2O$ of 0.75 normality caused less elution of 2,4-D adsorbed on Amberlite IR-100 H than did water. The amounts eluted by hydrochloric acid and water

were approximately equal. Solutions of NaCl, Na_2SO_4, and $Na_3PO_4 \cdot 12\ H_2O$ of 0.685 normality also eluted less 2,4-D from Amberlite IR-100 H than did water. Isopropyl N-phenylcarbamate, however, was eluted from IR-100 H by sodium chloride solution.

5. Hydrochloric acid eluted 2,4-D from anion exchangers, probably in typical anion- or acid-exchange reactions.

6. 2,4-D was mixed with Decalso and Zeo-Dur, respectively, at rates of 19.4 or about 64 mg. per pound. The mixtures were leached with three 2-inch columns of water. Much 2,4-D was removed by the first leaching, a smaller amount at the second leaching, and practically none at the third leaching.

7. About 93 micro-equivalents of six different growth-regulators were mixed with Decalso and Zeo-Dur and the mixtures were leached with a 2-inch column of water. One-half or more of each compound was usually leached from the exchanger materials, and the different compounds were usually removed in about equivalent amounts.

8. Growth of plants in soils containing ion exchangers upon which $NH_4$2,4-D was adsorbed showed that much of adsorbed growth-regulator is nontoxic. Barley and white mustard plants grew well in gravel–Zeo-Karb H mixtures containing $NH_4$2,4-D at concentrations of 100 and 10 mg., respectively, per pound of mixture. The growth-regulator may not have come into contact with the roots growing around the adsorbent or perhaps could not diffuse into or enter the plants.

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EMBRYOLOGY OF CEPHALOSTIGMA SCHIMPERI

S. B. KAUSIK AND K. SUBRAMANYAM

Introduction

The genus *Cephalostigma* A. DC. belongs to the family Campanulaceae, tribe Campanuloideae, subtribe Wahlenbergineae (4). GAMBLE (5) records three species in South India: *C. schimperi* Hochst., *C. flexuosum* Hook., and *C. hookeri* C. B. Clarke. The present investigation deals with *C. schimperi* only.

The literature on the embryology of the family has already been reviewed by SCHNARF (14) and by KAUSIK and SUBRAMANYAM (9) and need not be covered again.

Material and methods

The material was collected from three sources: Government Botanical Gardens, Lalbagh, Bangalore; Bannerghatta (near Bangalore); and Nandi Hills (a hill station 35 miles from Bangalore). It was fixed in Allen's modification of Bouin's fluid and in formalin–acetic-alcohol, both of which were quite satisfactory. Sections cut at a thickness of 10–20 μ were stained in Heidenhain's iron-alum haematoxylin with eosin as a counterstain.

Investigation

MICROSPOROGENESIS AND MALE GAMETOPHYTE

The wall of the anther (fig. 1) is made up of three layers external to the tapetum. The outermost is the epidermis; next is the endothecium, which acquires the usual fibrous thickenings in later stages; and then an ephemeral layer which is soon disorganized. The tapetal cells are at first uninucleate but later become binucleate and also begin to show conspicuous vacuoles.

The microspore mother cells undergo the usual reduction divisions and form tetrads of microspores (figs. 2–4) which are usually tetrahedral but sometimes show an isobilateral arrangement. The separation of the microspores takes place by cleavage furrows.

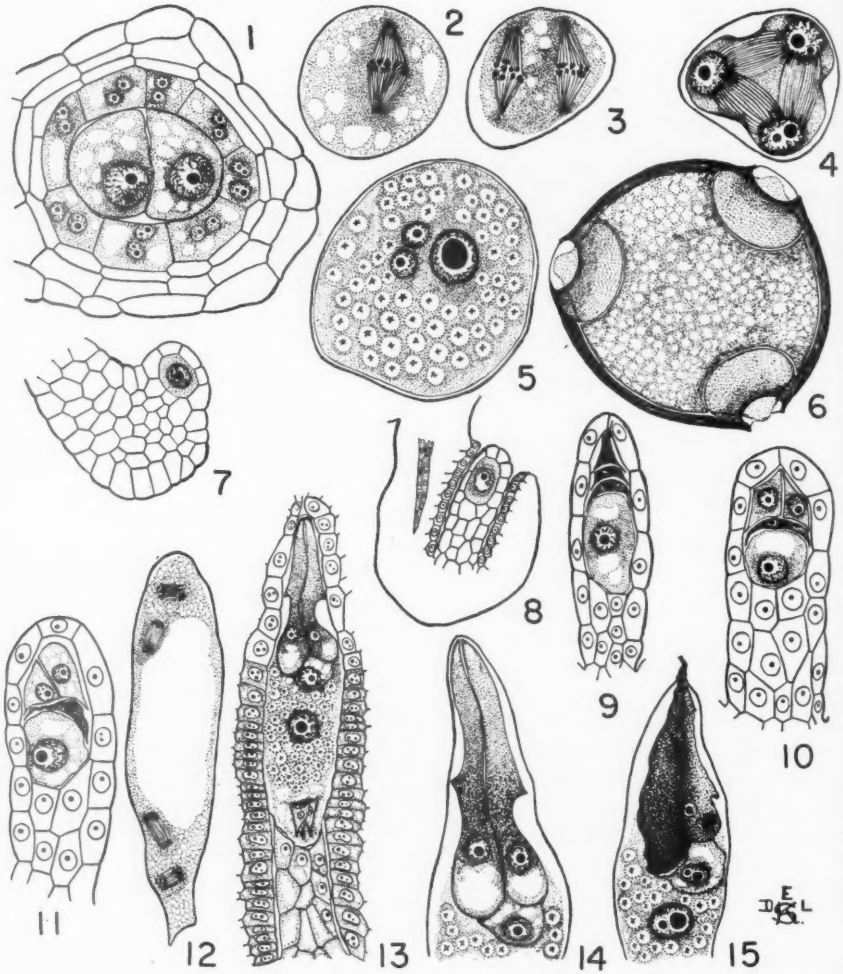
The mature pollen grain is packed with starch grains and contains three nuclei—a large tube nucleus and two small male nuclei (fig. 5). The exine is thick and rigid and shows reticulate thickenings on the surface (fig. 6). The intine appears as a thin delicate membrane. There are three conspicuous germ pores.

MEGASPOROGENESIS AND FEMALE GAMETOPHYTE

The ovary is inferior, tricarpellary, and trilocular, with numerous anatropous unitegmic ovules attached to axile placentae. The single hypodermal archesporial cell (fig. 7) functions directly as the megaspore mother cell (fig. 8). The megaspores may show a linear (fig. 9) or T-shaped (fig. 10) arrangement, and sometimes the micropylar dyad cell divides by an oblique wall (fig. 11). The three micropylar megaspores degenerate, and the chalazal one functions to give rise to a normal eight-nucleate embryo sac (figs. 12, 13).

The integument is well developed even at the megaspore mother cell stage (fig. 8). When the embryo sac is two- or four-nucleate, the nucellar epidermis becomes disorganized and the embryo sac comes in direct contact with the integumentary tapetum, which reaches its maximum development at the time of fertilization.

Meanwhile certain cells in the chalazal region of the nucellus become elongated

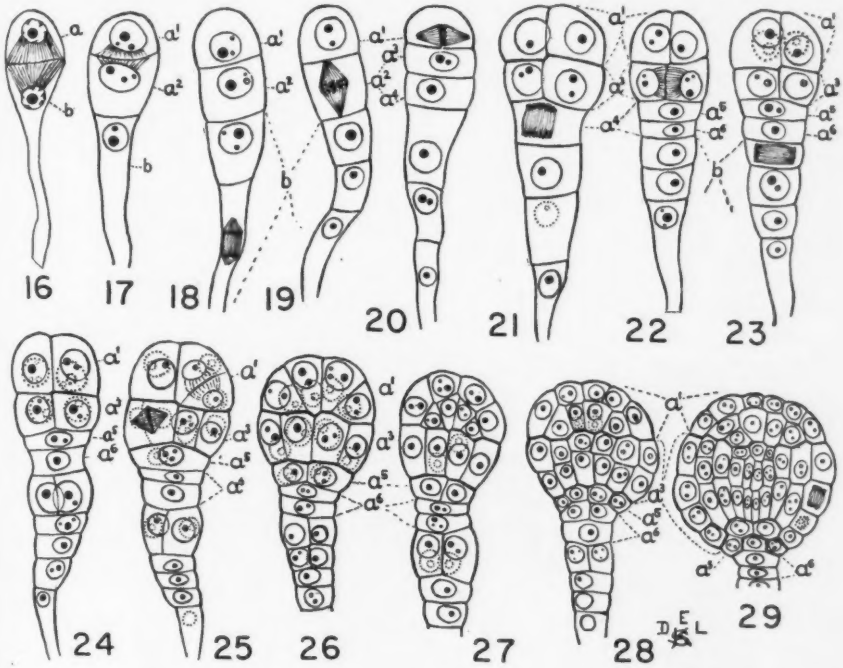


FIGS. 1–15.—Fig. 1, portion of cross section of young anther showing wall layers, binucleate tapetum, and microspore mother cells. ×900. Figs. 2, 3, 4, tetrad divisions in microspore mother cell. ×1800. Fig. 5, mature pollen grain showing trinucleate condition. Note starch grains in cytoplasm. ×1800. Fig. 6, pollen grain in surface view showing three germ-pores and reticulate exine. ×1800. Fig. 7, young ovule showing hypodermal archesporial cell and primordium of integument. ×600. Fig. 8, anatropous ovule showing megaspore mother cell. ×280. Fig. 9, linear tetrad of megaspores, the chalazal cell enlarging. ×900. Fig. 10, T-shaped tetrad of megaspores. ×900. Fig. 11, megaspore tetrad with oblique wall in upper dyad cell. ×900. Fig. 12, third nuclear division from megaspore leading to formation of eight-nucleate stage. ×900. Fig. 13, mature embryo sac showing egg apparatus, antipodal cells, and fusion nucleus; note starch grains. ×630. Fig. 14, upper part of embryo sac showing elongated synergids and egg cell. ×900. Fig. 15, stage in double fertilization showing remnants of pollen tube. ×900.

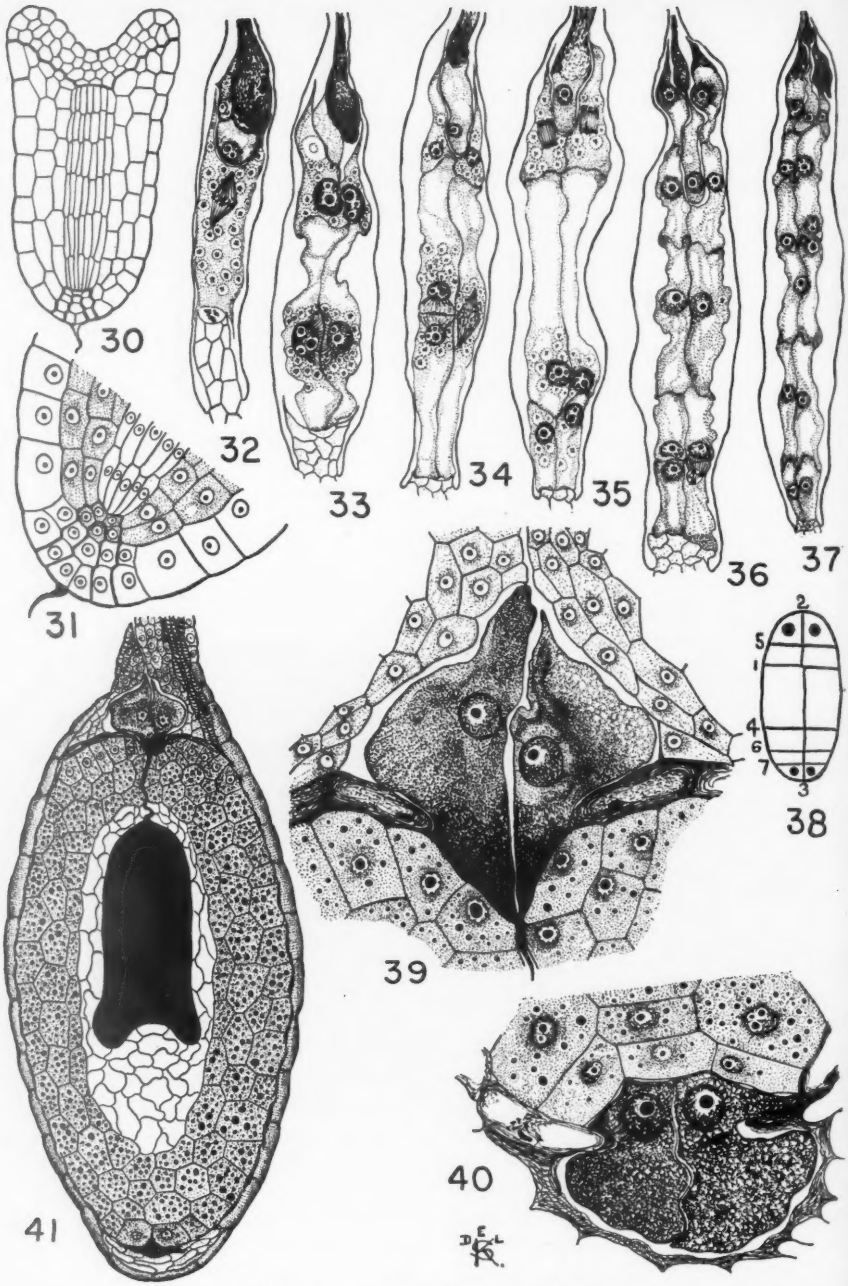
and thickened and seem to serve a conducting function (figs. 8–11, 13).

The fully mature embryo sac is tapering at both ends (fig. 13). The synergids are elongated structures with pointed beaklike apices. The pear-shaped egg cell is situated between them and has a conspicuous nucleus. The two polar nuclei meet near the center of the embryo sac and fuse to form the fusion nucleus. The antipodal cells taper at their lower ends and thus offer a marked resemblance to those of the allied family Lobeliaceae (**8, 9, 10**). They degenerate at about the time of fertilization. In another member of the same family, *Sphenoclea zeylanica* (**12**), and in one of the Lobeliaceae, *Lobelia nicotianaefolia* (**11**), this degeneration is postponed to a slightly later stage and takes place during the early stages of endosperm development.

A characteristic feature of the embryo sac is the presence of a large number of starch grains. Usually they appear after the fusion of the two polar nuclei (figs. 13–15) and persist during the early stages of endosperm development, being crowded around the nucleus of each endosperm cell (figs. 32–35). DAHLGREN (**2, 3**), who has reviewed the occurrence of starch grains in embryo sacs, lists *Campanula rotundifolia* as an instance where the starch appears at the two-nucleate stage but becomes considerably reduced in quantity in older stages.



FIGS. 16–29.—Stages in development of embryo. Primary segmentation walls are indicated by thicker lines. All ×900, except figs. 28, 29, ×630.



FIGS. 30–41.—Fig. 30, embryo showing cotyledons; note collapsed suspensor. ×270. Fig. 31, basal portion of mature embryo enlarged to show root-cap, dermatogen, periblem (stippled), and plerome. ×270.

(Legend continued on following page)

The pollen tube enters the embryo sac by destroying one of the synergids. Double fertilization takes place normally (fig. 15).

Embryo

The development of the embryo closely corresponds to that described for *Campanula patula* (15), *Lobelia syphilitica* (1), and *L. amoena* (6). Stages in development are presented in figures 16–31.

Endosperm

The primary endosperm nucleus, which lies near the zygote, divides (fig. 32), and the accompanying development of a transverse wall gives rise to a small primary micropylar and a larger primary chalazal chamber. Next a vertical wall is formed in each of these chambers, resulting in a four-celled stage (fig. 33). A transverse division now follows in the chalazal pair of cells (fig. 34) and subsequently in the micropylar pair (fig. 35), resulting in the formation of four tiers of paired cells. The two cells of the first tier now form the micropylar haustorium, and those of the lowest tier give rise to the chalazal haustorium after undergoing one transverse division (fig. 36) or perhaps two (fig. 37). The remaining tiers of cells, which lie between the haustoria, undergo further transverse and longitudinal divisions and give rise to the endosperm. Thus the sequence of wall formation (fig. 38) closely corresponds with that in the "Codonopsis type" found in other members of the Campanulaceae (13).

The micropylar haustorium (fig. 39) is two-celled, each cell forming a prominent lateral hump and containing a conspicuous nucleus imbedded in a dense mass of finely vacuolate cytoplasm. Being situated close to the rich nutritive tissue of the micropylar part of the integument, this haustorium remains active for a long time. The chalazal haustorium (fig. 40) consists of two bulbous cells which digest their way into the chalazal tissues and are later seen as darkly stained, compressed structures lying in a mass of collapsed cells.

All the cells of the endosperm, except those lying in the immediate vicinity of the embryo, contain consider .ble quantities of starch. The seed coat is formed by the outermost layer of the integument. In mature seeds (fig. 41) the embryo, with its shriveled suspensor, is seen buried in a large mass of endosperm.

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Fig. 32, first division of primary endosperm nucleus. ×630. Figs. 33–37, stages in development of endosperm and differentiation of micropylar and chalazal haustoria. Note aggregation of starch grains around nuclei of endosperm cells in figs. 32–35. Figs. 33–35, ×630; fig. 36, ×450; fig. 37, ×270. Fig. 38, diagram showing sequence of wall formation in endosperm. Fig. 39, two-celled micropylar haustorium in advanced stage. ×900. Fig. 40, two-celled chalazal haustorium in advanced stage. ×900. Fig. 41, longitudinal section of mature seed, showing embryo with collapsed suspensor, starchy endosperm, persisting micropylar haustorium, remnants of chalazal haustorium, and thick-walled seed coat. ×200.

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EFFECT OF A TEMPERATURE GRADIENT ON DISTRIBUTION OF WATER IN APPLES[1]

MILTON A. LESSLER

Introduction

Earlier work on apples and other fruits subjected to a temperature gradient has shown that there is water movement from the warmer to the cooler side (3, 4). Visible evidence of this movement is a progressively increased wrinkling and then a necrosis of the warmer side, while there is an increase in the turgidity of the cooler side. In some cases, the cool side may become turgid enough to split the skin.

LORENZE and KNOTT (5) showed that gray-wall or thin-wall of tomato occurred only on the side of the fruit that was exposed to the sun. They found that the condition resulted from a lowered water content on the exposed side, leading to the development of very small parenchyma cells on the inside of the pericarp. Temperature determinations of exposed and unexposed sides of the fruits *in situ* showed differences of as much as 15°–20° F. between the two sides.

REED and BARTHOLOMEW (6) made observations on young fruits of lemons, walnuts, apples, and tomatoes and found no intercellular spaces, but their observations led them to conclude that water passes over the surfaces of the cells as well as through them by osmosis. They concluded that in young fruits movement of water as a vapor is improbable but that water may move by capillarity through the hydrophyllic colloids present in the cell walls.

ARTHUR (1) attributed the shriveling

[1] Work conducted at Cornell University, Department of Botany, under the direction of Dr. O. F. CURTIS.

and necrosis, in apples, to a specific infra-red injury but later (2) stated that it is possible that an equal amount of energy in the visible region would produce a similar injury. CURTIS (3), using methods similar to those of ARTHUR (1), found that the wrinkling and later necrosis of the warm side of the apple resulted from a movement of water from the warmer to the cooler side. Further studies on this problem will be presented in this report.

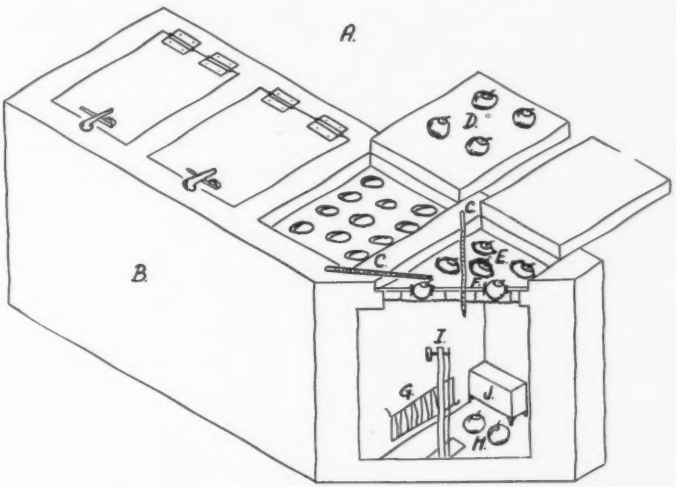

FIG. 1.—Apparatus used for gradient treatment. *A*, 6° C. refrigerator room; *B*, incubator; *C*, thermometers; *D*, cold control apples; *E*, asbestos panel with experimental apples; *F*, cotton packing around apple; *G*, heating unit; *H*, warm control apples; *I*, thermostat; *J*, thermograph.

Experimentation

The apparatus for producing a temperature gradient was an incubator placed on its back, so that the doors were on the upper side (fig. 1), in a constant-temperature room kept at 6° C. The incubator doors were laid back, and carefully fitted asbestos panels were put into the openings. The fruits were fitted into holes in the panels, and cotton was carefully packed around them.

All fruits were weighed before and after the gradient treatment and the total water loss noted. This loss varied but averaged only 28% of the actual difference between the cool and warm sides of the fruits and was of the same order as the total water loss from the controls.

After treatment, each fruit was cut into warm and cool halves on a premarked line and each half put on a marked tared watch glass. After drying for 7 days in a blower oven at 65° C. and for 7 days in a vacuum oven at 60° C. the samples were again weighed. This drying treatment brought the halves to constant weight.

Figure 2 graphically shows the results from twenty-eight apples (variety Wealthy) given gradient treatment and eleven apples used as controls. The air on the warm side was kept at 25° C. and on the cool side at 6° C. Results from representative apples taken at intervals indicated a rapid movement of water (from

the warmer to the cooler side) for the first 3 days and then a tendency for the rate of movement to drop off.

In a determination of the relative distribution of water in apples after a $3\frac{1}{2}$-day gradient treatment with the air on the warm side at 20° C. and on the cool side at 6° C., plugs 1 cm. in cross section were cut both parallel and perpendicular to the stem-flower axis; each plug was cut transversely into three parts of equal length. To compensate for differences in water content that might occur naturally between stem and flower ends of the fruit, some had been placed in the apparatus stem up, others stem end down, and others with the stem-flower axis horizontal. Table 1 summarizes the data obtained from plugs from treated and control apples. Those from apples given a gradient treatment showed a distribution of water which was lowest on the warm side, highest in the middle, and next highest on the cool side. Statistical treatment indicated that the differences in percentage of water between warm and cool and between warm and middle samples were significant but that the difference between cool and middle sections was not. Since the highest percentage of water was found in the middle portion of both experimental and control plugs, it is reasonable to assume that it was higher there at the start of the experiment, possibly because the two end portions of the plugs had skin on them, while there was none on the middle section.

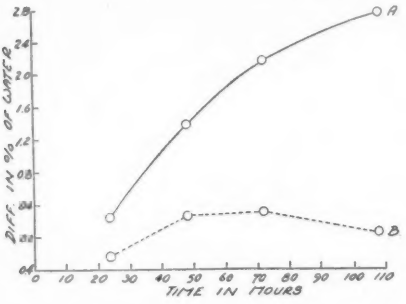

FIG. 2.—Movement of water in apples during $4\frac{1}{2}$-day gradient treatment. *A*, difference in % of water between cool and warm halves of experimental apples; *B*, difference in % of water between halves of control apples.

Young Wealthy and Oldenburgh apples ($1-1\frac{7}{8}$ inches in diameter) were given a $3\frac{1}{2}$-day gradient treatment (cool side 6° C., warm side 25° C.). Visual observations, after the treatment, showed some wrinkling of the warmer sides; the cooler sides were somewhat more turgid than the controls. There was a small but measurable difference in percentage of water between the warmer and cooler sides of the fruits, but the data were not clearly significant when treated statistically. The differences noted, in this case, may have resulted from the warm side losing more water by evaporation. Microscopic observations of freehand sections of the succulent tissue of these young fruits showed only occasional intercellular spaces. When portions of the fruits were placed in a dilute solution of India ink in a suction flask which was

TABLE 1

DISTRIBUTION OF WATER AFTER 84-HOUR GRADIENT TREATMENT. AIR TEMPERATURE ON WARM SIDE, 20° C.; ON COOL SIDE, 6° C. (TESTED BY PLUG METHOD)

	Experimental	Control*
Number of samples	20	6
Water % fresh wt. cool side	85.56	84.53
Water % fresh wt. middle	85.84	84.94
Water % fresh wt. warm side	84.36	84.18
Cool minus warm	1.20	0.35
Middle minus warm	1.48	0.76
Middle minus cool	0.28	0.41

* For controls both sides were at same temperature but one side was marked *a*, the other *b*. Differences are given as *a* minus *b*, middle minus *b*, and middle minus *a*.

evacuated during 30 minutes, there was almost no penetration of the ink into the tissue except near the edges where the cells had been torn in the cutting.

Fruits on the tree are subjected to a marked temperature gradient only during the time that the sun strikes their unshaded side. An attempt to duplicate the effect of such an alternating gradient is shown graphically in figure 3. Experimental apples were given a 12-hour gradient treatment (cool side 6° C., warm side 35° C.) and were then freely sus-

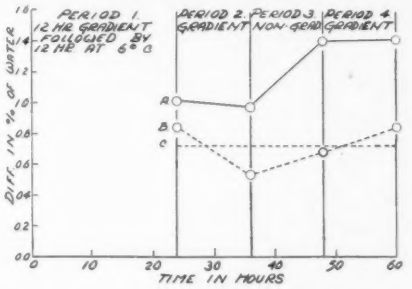

FIG. 3.—Movement of water in apples during alternating periods of gradient and nongradient treatment. *A*, difference in % of water between cool and warm halves; *B*, difference in % of water between stem and floral halves of controls; *C*, arithmetic average of curve *B*.

pended in the cold room for 12 hours. This alternating treatment was carried out for 2½ days, with samples taken at the end of each 12-hour period after the first 24 hours. Controls were kept both in the incubator and in the cold room.

In figure 3 the small movement of water from warm to cool side in period 1 and the apparent reverse movement during period 2 may be accounted for by excessive evaporation from the fruits during the early part of the treatment. This makes it difficult to draw conclusions; however, a comparison of curve *A* with *B* or *C* indicates that there was movement of water from the warmer to the cooler side of the experimental fruits during the gradient treatment and during nongradient (period 3). The latter is explained by the fact that after removal from the gradient treatment there is a period of time before the fruit comes to a uniformly constant temperature, and water may continue to move during that time.

Thermocouples were used to measure the differences in temperature between the shaded and sunny sides of Wealthy apples naturally attached to the tree. Temperature readings were taken near noon on almost cloudless days in August when the air temperature was 28°–32° C.

TABLE 2

LARGEST TEMPERATURE DIFFERENCES BETWEEN TWO SIDES OF WEALTHY APPLES DURING 1-HOUR TEST PERIOD UNDER NATURAL CONDITIONS (AIR TEMPERATURE 28°–32° C.)

Shady Side	Sunny Side	Gradient
30.0° C.	39.5° C.	9.5° C.
30.6	40.1	9.5
31.1	40.8	9.7

Table 2 gives the largest differences found during a 1-hour test period, showing that under natural conditions a 9.7° C. temperature gradient may occur. CURTIS (3) showed that, if such a gradient is maintained, water will move from the warmer to the cooler side of the fruit.

Discussion

The decrease in rate of movement of water across the apples, after the first 3 days of gradient treatment, probably resulted from the water being held more strongly by the osmotic and imbibitional forces of the desiccated side. In young apples the small amount of water movement may be explained on the basis of their lack of intercellular spaces, which limits the chances for movement of water by distillation; it is possible that the

water moved by capillarity through the hydrophyllic colloids of the cell walls.

In a natural situation one would expect to find an alternating temperature gradient. When the sun strikes a fruit only on one side, that side may become from 10° to 18° F. warmer than the shaded side. When a fruit is removed from such a situation, it is reasonable to assume that there will be a period of time before the fruit comes to a uniformly constant temperature. During that time water will continue to move from the warmer to the cooler side. After the fruit has reached a uniformly constant temperature, the imbibitional and osmotic forces of the more desiccated side are not strong enough to reverse the process to any extent.

Summary

1. Mature apples (variety Wealthy) exposed to a temperature gradient show a fairly rapid rate of water movement across the fruit (from the warmer to the cooler side) for the first 3 days and then a slower rate of movement. Gradient treatment of apples caused significant amounts of water to move across the fruits in less than 48 hours.

2. After a $3\frac{1}{2}$-day gradient treatment plugs from mature apples showed a greater percentage of water in the middle, next highest on the cool side, and lowest on the warm side. The controls also showed a higher percentage of water in the middle.

3. After 3 days of treatment a small but measurable difference in percentage of water was found between the warm and cooler sides of young ($1-1\frac{7}{8}$ inch diameter) apples.

4. Mature apples subjected to alternating 12-hour gradient and nongradient treatments showed water movement from the warmer to the cooler sides.

5. Wealthy apples on the tree, during a sunny summer day, showed a maximum temperature gradient of 9.7° C. between the sunny and the shaded sides.

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